

# 6<sup>th</sup> JOINT CONFERENCE OF DGHM & VAAM

**8–11  
March  
2020  
Leipzig**

**ABSTRACTS**



**72<sup>nd</sup> Annual Meeting**

German Society for  
Hygiene and Microbiology



**Annual Meeting 2020**

Association for General and  
Applied Microbiology

## 001-EMV

Impact of ocean acidification on microbial eukaryotes from the Timor Sea

\*J. Rahlff<sup>1,2</sup>, S. Khodami<sup>3</sup>, L. Voskuhl<sup>2</sup>, M. P. Humphreys<sup>4,5</sup>, C. Stolle<sup>1,6,7</sup>, P. M. Arbizu<sup>3</sup>, M. Ribas-Ribas<sup>1</sup>

<sup>1</sup>*Institute for Chemistry and Biology of the Marine Environment (ICBM), Carl von Ossietzky University Oldenburg, Wilhelmshaven, Germany*

<sup>2</sup>*Institute for Environmental Microbiology and Biotechnology, University of Duisburg-Essen, Essen, Germany*

<sup>3</sup>*Senckenberg am Meer, German Center for Marine Biodiversity Research, Wilhelmshaven, Germany*

<sup>4</sup>*Ocean and Earth Science, University of Southampton Waterfront Campus, National Oceanography Centre Southampton, Southampton, United Kingdom*

<sup>5</sup>*NIOZ Royal Netherlands Institute for Sea Research, Utrecht University, Department of Ocean Systems (OCS), Texel, Netherlands*

<sup>6</sup>*Leibniz Institute for Baltic Sea Research (IOW), Rostock, Germany*

<sup>7</sup>*Project management Jülich, Rostock, Germany*

## Introduction

Increased anthropogenic carbon dioxide (CO<sub>2</sub>) emissions drive climate change and pose one of the major challenges of our century. CO<sub>2</sub> effects in the form of ocean acidification (OA) on the communities of marine microbial eukaryotes from the Timor Sea are barely understood.

## Objectives

The study objective was to reveal effects of high CO<sub>2</sub> versus *in situ* CO<sub>2</sub> seawater on the community composition of therein living marine microbial eukaryotes immediately and after 48 hours of treatment exposure.

## Materials & methods

In a shipboard microcosm experiment, seawater from 65 m depth of the Timor Sea was acidified in bottles to pH=7.6 corresponding to a  $p\text{CO}_2=1668 \mu\text{atm}$  and compared to *in situ* conditions at pH=8.1 and  $p\text{CO}_2=463 \mu\text{atm}$  (both  $n=3$ ). After filtration and nucleic acid extraction from time zero and after 48 hours, Illumina sequencing of the V9 hypervariable region of 18S rRNA (gene) was used to study the eukaryotic community composition. In addition, chlorophyll *a* content, and the concentration of extracellular carbonic anhydrase, an enzyme with a role in concentrating CO<sub>2</sub>, were measured.

## Results

Down-regulation of a carbon concentrating mechanism within 48 hours occurred faster in the high CO<sub>2</sub> treatment. We detected significant suppressing effects by OA, compared to *in situ* conditions on the eukaryotic operational taxonomic units (OTUs), including diatoms and haptophytes. These effects were often consistent between abundant (DNA-based) and active (cDNA-based) taxa after 48 hours, e.g. for the diatoms *Trieres chinensis* and *Stephanopyxis turris*, and also applied for OTUs with rel. abundance <0.01%. A bottle incubation effect aligned the abundance of abundant and active OTUs. It further allowed certain taxa to flourish and to study their response to the high CO<sub>2</sub> treatment.

## Conclusion

The microbial eukaryotes showed some adaptation to the CO<sub>2</sub> treatment over time, but many OTUs were adversely affected by OA-driven seawater pH changes. While effects on rare taxa are particularly prone to be overlooked, OA effects might fundamentally impact on the basis of marine

biodiversity implicating unpredictable outcomes for food web functioning in a future ocean.

## 002-EMV

### Microbial community response to submarine permafrost warming and thaw

\*J. Mitzscherling<sup>1</sup>, F. Horn<sup>1</sup>, J. Kallmeyer<sup>1</sup>, P. P. Overduin<sup>2</sup>, M. Winkel<sup>3</sup>, M. N. Grigoriev<sup>4</sup>, D. Wagner<sup>1,5</sup>, S. Liebner<sup>1,6</sup>

<sup>1</sup>*GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Geomicrobiology, Potsdam, Germany*

<sup>2</sup>*Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Permafrost Research, Potsdam, Germany*

<sup>3</sup>*GFZ German Research Centre for Geosciences, Helmholtz Centre Potsdam, Interface Geochemistry, Potsdam, Germany*

<sup>4</sup>*Russian Academy of Sciences, Mel'nikov Permafrost Institute, Siberian Branch, Yakutsk, Russian Federation*

<sup>5</sup>*University of Potsdam, Institute of Geosciences, Potsdam, Germany*

<sup>6</sup>*University of Potsdam, Institute of Biochemistry and Biology, Potsdam, Germany*

Global warming leads to permafrost thawing which stimulates microbial carbon turnover and the formation of greenhouse gases. Permafrost does not only warm and thaw on land but also on the Arctic shelf where submarine permafrost covers about three million km<sup>2</sup>. Yet, very little is known about how microbial communities respond to warming and thawing of permafrost deep below the seafloor.

We studied microbial community abundance and composition in submarine permafrost relative to onshore permafrost with a focus on the microbial community response to long-term submarine permafrost warming and seawater penetration.

The microbial abundance as a measure of total cell counts and quantitative PCR, as well as community composition and diversity based on 16S rRNA Illumina sequencing were investigated in permafrost cores from two locations in the Siberian Laptev Sea, where permafrost had been subjected to submarine conditions for centuries to millennia. Molecular biological analyses were related to permafrost temperature, pore water composition, and paleo-climatic proxies in order to identify which of these factors control the response of permafrost microbial communities to submarine conditions.

The combination of centennial permafrost warming and seawater infiltration resulted in a loss both of microbial abundance and bacterial diversity. At the same time, the bacterial community of warmed permafrost without thaw was still determined by the environmental and climatic conditions at the time of sediment deposition rather than by the temperature increase over centuries. A stimulating effect of permafrost warming was reflected in an increased microbial abundance and in reduced amounts of substrate only millennia after sea water inundation.

In summary, despite the exposure to submarine conditions for centuries to millennia, the bacterial permafrost community generally resembled that of terrestrial permafrost without a clear influence on it due to submarine permafrost warming.

## 003-EMV

Abundance and community composition of methanotrophic bacteria depend on small spatial scale changes in an arctic-alpine system

\*K. Frindte<sup>1</sup>, J. Löffler<sup>1</sup>, C. Knief<sup>1</sup>

<sup>1</sup>*University of Bonn, Molecular Biology of the Rhizosphere, Bonn, Germany*

Arctic-alpine ecosystems show a high spatial heterogeneity, which is related to differences in elevation and micro-topographic features like slopes, ridges and depressions. We hypothesize that this spatial variation supports different habitats for aerobic methanotrophic microorganisms due to changes in soil moisture and temperature regimes. We extracted DNA from 96 soil samples taken from two mountain regions in the Norwegian Scandes with strong gradients in micro-topography and elevation. Quantitative PCR and amplicon sequencing of the functional marker gene *pmoA* was used to investigate differences in methanotrophic abundance and community composition. To explore the effect of long-term temperature and soil moisture regimes on the methanotrophic communities, partial least square regression (PLSR) analysis was performed. First results indicate that the *pmoA* copy number decreases in depressions with increasing elevation, but this elevational trend was not observed within other micro-topographic locations. PLSR analysis showed that the main driver for variation in methanotrophic abundance was the long-term mean soil moisture, recorded over a period of five years. The methanotrophic community composition between sites was similarly affected by elevation and micro-topography (ANOSIM,  $R=0.28$ ,  $P < 0.001$  for both factors). Phylogenetic identification revealed that high abundant members ( $> 1\%$  relative abundance) of the upland soil cluster (USC)  $\alpha$  increased in relative abundance with elevation. Moreover, USC  $\alpha$  OTUs were more abundant at ridges, while depressions were dominated by Cluster 5 and *Methylocystis* strains. PLSR with the community compositional data are ongoing to assess the relevance of soil factors (pH, organic carbon, nitrogen content), long-term temperature and soil moisture conditions on the methanotrophs in this ecosystem. Our data demonstrate that the distribution of methanotrophic bacteria is spatially very variable in an arctic-alpine ecosystem and suggest that soil moisture is likely a major driving factor for this pattern.

#### 004-EMV

##### Skin microbiome composition of three different frog species and distribution of a pathogenic fungus in Pyrenean lakes

\*A. Cravo<sup>1,2</sup>, R. Kallies<sup>1</sup>, A. Loyau<sup>2,3</sup>, D. Schmeller<sup>2</sup>, A. Kuppardt-Kirmse<sup>1</sup>, A. Chatzinotas<sup>1,4</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology (UMB), Leipzig, Germany

<sup>2</sup>Université de Toulouse, CNRS, INPT, UPS, ECOLAB, Toulouse, France

<sup>3</sup>Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Experimental Limnology, Berlin, Germany

<sup>4</sup>German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

The major threats for amphibian population declines are habitat degradation, climate change and spread of pathogens, such as the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*). Amphibian skin bacteria may play a crucial role in protecting their host against pathogens. However, current findings vary from study to study. For example, we are largely oblivious if anthropogenic factors alter the amphibian skin microbiome and thus its potentially protective role. In this study, we compared the skin microbiome composition of *Bd*-infected tadpoles with non-infected individuals for the species *Alytes obstetricans* using 16S rRNA (bacteria) and 18S rRNA (micro-eukaryotes) gene based high throughput sequencing (Illumina MiSeq). Individual *Bd* infection state of *A. obstetricans* tadpoles was determined using qPCR. In addition, we also compared the skin microbiome of *Alytes obstetricans* tadpoles with the skin microbiome of *Rana temporaria* and *Bufo bufo* in 20 different Pyrenean lakes. These data were analyzed in regard to different anthropogenic pressures (such as touristic impact,

livestock and presence of fish) and temporal variability (beginning, middle and end of summer). Our results show that seven out of 20 lakes were infected with *Bd*, with, at least, 10% prevalence on the sampled individuals. We found a significant difference in the bacterial richness between the three species across the lakes ( $p=0.01$ ) with posterior pairwise comparison showing a more strong difference between *Rana temporaria* and *Alytes obstetricans* ( $p<2e-6$ ). Differences were also detected between infected and non-infected individuals of *A. obstetricans* ( $p=0.01$ ) combining the data of the three periods. The most frequent genus was *Limnohabitans*, a known freshwater bacterial taxon, which was present in 55% of all samples and which was more abundant in *A. obstetricans* and *R. temporaria* than in *B. bufo*. The first results show a higher influence of the host and the environment on the microbiome composition than of the presence of *Bd* on *A. obstetricans* samples. We will further discuss the bacterial dataset in the context of environmental factors and the *Bd* distribution.

#### 005-EMV

##### Extended-spectrum beta lactamase (ESBL)-producing *E. coli* released with manure and biogas plant digestates into the environment

O. E. Aigbekaen<sup>1</sup>, H. M. M. Khan<sup>1</sup>, \*S. P. Glaeser<sup>1</sup>, P. Kämpfer<sup>1</sup>

<sup>1</sup>Justus Liebig University, Applied Microbiology, Giessen, Germany

Extended-spectrum beta lactamase (ESBL) producing *Escherichia coli* are a health care problem today. Intensive application of antibiotics in livestock increases the release of ESBL *E. coli* via manure into soil, ground and surface waters. Beside the direct application of manure as fertilizers on fields, manure is anaerobically digested in small scale biogas plants in Germany which should reduce the abundance of ESBL *E. coli* in digestates and subsequently their release to the environment if the digestate is used for fertilization. As part of the international JPI-EC-AMR collaborative project Antimicrobial Resistance Manure Intervention Strategies (ARMIS), we study the abundance and antibiotic resistance patterns of ESBL *E. coli* in both manure and biogas plant digestates by a cultivation approach. So far input and output samples of five German Biogas plants (four mesophilic and one thermophilic) were investigated. ESBL *E. coli* were cultured on TBX agar with 4 mg L<sup>-1</sup> cephalosporins which was incubated for four hours at 37°C and 20 hours at 44°C. In addition a pre-enrichment was performed according to Schauss et al. (2015) to enhance the detection efficiency. ESBL *E. coli* were cultured by direct plating from manure and after pre-enrichment also from output material. Genomic fingerprinting showed the presence of the same genotypes in input and output samples indicating a transfer of ESBL *E. coli* through the biogas plants. The strains contained blaCTXM and blaTEM genes. Whether or not differences in the antimicrobial resistance pattern is caused by anaerobic digestion, has to be further investigated.

#### 006-EMV

##### Dissemination of antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes through hospital wastewater – a weekly profile

\*E. Sib<sup>1</sup>, C. Felder<sup>1</sup>, A. M. Voigt<sup>1</sup>, F. Lenz-Plet<sup>1</sup>, H. Färber<sup>1</sup>, M.

Exner<sup>1</sup>, D. Skutlarek<sup>1</sup>, R. M. Schmithausen<sup>1</sup>

<sup>1</sup>University Clinic Bonn, Institute for Hygiene and Public Health, Bonn, Germany

#### Introduction

Bacteria and chemical pollutions are indicators for the ecological health of water bodies. In recent years antibiotic resistant bacteria, as well as antibiotic residues and antibiotic resistance genes have disseminated to surface waters. Those parameters play a crucial role in the healthcare system due to the life threatening risk multidrug resistant bacteria pose to humans.

## Objectives

The aim of this study was to depict a weekly profile of antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in wastewater of a maximum care hospital to monitor the dissemination of these parameters into the environment.

## Materials & methods

In total 504 one hour mixed wastewater samples were taken to build a weekly profile of the researched clinic using an automated sampler. All water samples were analyzed for antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes. Quantitative analysis of 43 antibiotic residues was performed using LC-MS/MS. Resistance genes were examined using real time polymerase chain reaction (RT-PCR). Cultural analysis for multidrug resistant bacteria was performed using chromogenic selective agar plates (MRSA, VRE, ESBL) and morphological and physiological characteristics. The isolates were identified using MALDI-TOF MS and further characterized via microbroth dilution.

## Results

The examined hospital wastewater contained residues of clinically important antibiotics, for example meropenem and vancomycin in varying concentrations. Further, relevant antibiotic resistance genes (carbapenemases) were detected throughout the whole sampling period. In addition, antibiotic resistant bacteria were identified in all water samples, including resistant strains of *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Escherichia coli*.

## Conclusion

Hospital wastewater consists of high concentrations of clinically relevant antibiotic residues, highly resistant bacteria and high amounts of antibiotic resistance genes. To prevent the further dissemination and acquisition of resistance and to protect the health of society, a specific treatment of hospital wastewater is needed.

## 007-EMV

Rapid antibiotic susceptibility testing of *Enterobacterales* isolated from drinking water systems using MALDI-TOF mass spectrometry direct-on-target microdroplet growth assay (DOT-MGA)

S. Schwerthöffer<sup>1</sup>, \*I. D. Nix<sup>2</sup>, E. A. Idelevich<sup>2</sup>, O. Drews<sup>3</sup>, K. Sparbier<sup>3</sup>, M. Kostrzewa<sup>3</sup>, A. Bauwens<sup>1</sup>, H. Schünke<sup>1</sup>, K. Becker<sup>2,4</sup>

<sup>1</sup>Westfälische Wasser- und Umweltanalytik GmbH, Gelsenkirchen, Germany

<sup>2</sup>University Hospital Münster, Institut of Medical Microbiology, Münster, Germany

<sup>3</sup>Bruker Daltonik GmbH, Bremen, Germany

<sup>4</sup>University Medicine Greifswald, Friedrich Loeffler-Institute of Medical Microbiology, Greifswald, Germany

**Introduction** Multi-drug resistant microorganisms may pose an increasing threat to human health. Thus, accurate and rapid detection of their resistance patterns is required.

However, a standard method for susceptibility testing in drinking water analytics is still missing. The MALDI-TOF MS-based direct-on-target microdroplet growth assay (DOT-MGA) is a novel approach for rapid antibiotic susceptibility testing of bacteria and could become an alternative for testing microorganisms isolated from drinking water systems.

**Objectives** We aimed to establish a DOT-MGA approach for rapid AST of *Enterobacterales* isolated from drinking water systems, using adapted growth conditions for these isolates.

**Material and Methods** Isolates from drinking water systems were used. Overnight cultures were done on blood agar or yeast extract agar at 25°C or 35°C, respectively. Applying the new MBT FAST prototype kit (Bruker), DOT-MGA was performed with six clinically relevant *Enterobacterales*-active antibiotics at EUCAST breakpoint concentrations. In addition to cation-adjusted MH broth, nutrient broth (Oxoid) was used. Microdroplets of 6 µl were spotted in duplicates directly onto the spots of a MBT Biotarget 96 and incubated in a humidity chamber for 4.5, 8 and 24 h at 25°C or 35°C, respectively. Subsequently, medium was removed using absorbent pads and MBT FAST Matrix was added. Measurements were performed in duplicate using MBT FAST settings and spectra were evaluated with the MBT FAST prototype.

**Results** Overall, best test performance was achieved using nutrient broth at 35°C. Comparing the results of the DOT-MGA with those obtained by the standard broth microdilution according to EUCAST after 4.5, 8 and 24 h incubation, categorical agreements of 94, 96 and 96%, respectively, with test validity of 92, 92 and 75% were observed.

**Conclusion** This study indicated that rapid antibiotic susceptibility testing of *Enterobacterales* isolated from drinking water systems using the MALDI-TOF MS-based DOT-MGA is feasible and accurate within 4.5 h using special growth conditions. Standardization, improved spectrum evaluation and confirmation of this proof-of-principle study's results are needed.

## 008-EMV

Consortium of Indigenous Endophytic Bacteria for Control of Fusarium Wilt disease (*Fusarium oxysporum* f.sp *lycopersici*) and Promote Growth and Yield of Tomato (*Lycopersicon esculentum* Mill.)

\*Y. Yanti<sup>1</sup>, W. warnita<sup>2</sup>, H. Hamid<sup>1</sup>

<sup>1</sup>university, plant protection, Padang, Indonesia

<sup>2</sup>university, agrotechnology, Padang, Indonesia

*Fusarium oxysporum* f.sp *lycopersici* which causes wilt disease is one of serious detention in cultivating tomatoes which can cause losses up to 100%. Synthetic fungicides usage which usually used in controlling plant pathogens may caused resistance to these soil-borne pathogens, thus making it more difficult to control. Control by utilizing biological agents such as endophytic bacteria may become an alternative that can be developed. The purpose of this study was to acquire the best consortium from selected indigenous endophytic bacterial isolates that effectively control *Fusarium oxysporum* f.sp *lycopersici* and promote tomato growth and yields. This study was designed in experimental study using a completely randomized design. The treatment given was the introduction of indigenous endophyte bacteria consortium with 3 replications. The results obtained were by 6 consortiums of compatible endophytic bacteria to be combined. The consortiums 2,4, 5 and 6 were the best consortia that effectively controlled *Fusarium oxysporum* f.sp *lycopersici* with incidence reduced

up to 100% and were able to increase tomato yield by fruit weight 53.60 gr / plant.

Keywords: Endophytic bacteria, Consortium, Fusarium wilt

## 009-DKMV

Applicability and performance of EUCAST's rapid antibiotic susceptibility testing (RAST) on sterile body fluid in blood culture bottle

\*J. K. Jasuja<sup>1</sup>, S. Zimmermann<sup>1</sup>, I. Burckhardt<sup>1</sup>

<sup>1</sup>University Hospital Heidelberg, Department for Infectious Diseases, Heidelberg, Germany

### Introduction:

It is proven that the inoculation of sterile body fluid in blood culture bottle is beneficial for isolating more significant pathogens. EUCAST'S RAST has yet only been evaluated for positive blood culture bottles, so it is still unclear if RAST is also applicable on sterile body fluids in blood culture media.

### Objectives:

The objective of this study was to test the applicability of RAST on sterile body fluids in blood culture bottles and to evaluate its performance regarding category agreement and errors.

### Materials & methods:

Positive blood culture bottles (BACTEC™ Aerobic, BACTEC™ Anaerobic, BACTEC™ PED) inoculated with sterile body fluid (e.g. joint fluid, ascites, dialysate) from patients were subcultured onto Columbia 5% SB agar, chocolate agar, MacConkey agar, Schaedler agar (if anaerobic) and Mueller-Hinton agar. On latter, six commonly used antibiotic discs were added (cefotaxime, ampicillin, vancomycin, piperacillin/tazobactam, meropenem and ciprofloxacin). Subculture and streaking was done in the semi-automatic part of total lab automation (TLA). Subcultures and RAST were imaged after 6h and MALDI-TOF MS was performed with 6h growth. Zone sizes were measured using the ReadA Browser software of the TLA and interpreted according to RAST breakpoints for positive blood culture bottles (Version 1.0/1.1). MIC values were determined using VITEK2 panels for all isolates.

### Results:

In the time period of November 2018 to August 2019 *Staphylococcus aureus* (n=143), *Enterococcus spp.* (n=72) and *Escherichia coli* (n=33) were the most frequent detected pathogens. Comparison of RAST and MIC results showed 5.6% of major errors for *Enterococcus spp.* in Ampicillin and 3.9% in Vancomycin. 7.7% of minor errors were found for *E.coli*. Very major errors were not found for any pathogen. 3 and 18 samples were found with area of technical uncertainty (ATU) in gram-positive cocci and *E.coli*, respectively. Category agreement between MIC values and RAST was 94.6%.

### Conclusions:

Applicability and performance of RAST on sterile body fluid in blood culture bottle is promising and patients may benefit from the rapid procedure. Further investigation of ATU is recommended.

## 010-DKMV

MALDI-TOF MS-based optochin susceptibility testing for differentiation of *Streptococcus pneumoniae* from other *Streptococcus mitis* group streptococci

\*I. D. Nix<sup>1</sup>, E. A. Idelevich<sup>1</sup>, A. Schlattmann<sup>1</sup>, K. Sparbier<sup>2</sup>, M. Kostrzewa<sup>2</sup>, K. Becker<sup>1,3</sup>

<sup>1</sup>University Hospital Münster, Institut of Medical Microbiology, Münster, Germany

<sup>2</sup>Bruker Daltonik GmbH, Bremen, Germany

<sup>3</sup>University Medicine Greifswald, Friedrich Loeffler-Institute of Medical Microbiology, Greifswald, Germany

### Introduction

Differentiation of *Streptococcus pneumoniae* from other *S. mitis* group streptococci (SMGS) using MALDI-TOF mass spectrometry (MS) is still challenging. For identification of *S. pneumoniae*, optochin susceptibility test (OST) by disk diffusion is one of the methods recommended by the WHO. Since MALDI-TOF MS has become standard for microbial identification, using this technology even for this purpose would simplify routine workflow.

### Objectives

We aimed to develop a novel MALDI-TOF MS-based OST for differentiation of *S. pneumoniae* from other SMGS using the recently introduced MALDI-TOF MS-based direct-on-target microdroplet growth assay (DOT-MGA).

### Material and Methods

For test development, reference strains and clinical isolates (n=13) were used. To define a provisional breakpoint (BP) for optochin susceptibility, MICs were determined by broth microdilution method according to CLSI. For DOT-MGA, bacterial suspensions in cation-adjusted MH broth with lysed horse blood were added with or without 2, 4 or 8 µg/mL optochin in duplicates directly onto the spots of a disposable MBT Biotarget (Bruker) as microdroplets of 6 µl. The inoculated targets were incubated for 24h at 35°C in the incubation chamber (MBT FAST Shuttle). Subsequently, medium was removed using the stamp device and 1 µl formic acid was added onto dried spots, before adding MBT FAST Matrix. Measurements were performed in duplicate using MBT FAST settings.

### Results

Optochin MICs for *S. pneumoniae* and for *S. mitis* /*S. oralis* were in range 0.25-0.5 µg/mL and 8-128 µg/mL, respectively. Provisional optochin BPs of 2, 4 and 8 µg/mL were tested. Applying the DOT-MGA, successful detection of growth control (MALDI Biotyper score ≥1.7) was achieved in 100% of tests. 100% sensitivity and 100% specificity were achieved with all tested BP concentrations when Biotyper score ≥1.7 was defined as optochin-resistant and score

### Conclusion

MALDI-TOF MS-based OST is a suitable method to distinguish pneumococci from viridans streptococci. Standardization, automated spectrum evaluation and confirmation of this proof-of-principle study's results in further studies are needed.

## 011-DKMV

Automated antimicrobial susceptibility testing of common gram-negative pathogens – How exact are commercial systems?

\*J. Dick<sup>1</sup>, M. Marschal<sup>1</sup>, U. Schoppmeier<sup>1</sup>, S. Peter<sup>1</sup>

<sup>1</sup>University Hospital Tübingen, Institute of Medical Microbiology and Hygiene, Tübingen, Germany

Introduction: Antimicrobial susceptibility testing (AST) is one of the key challenges of clinical microbiology. Automated



systems are commonly used for high-throughput AST in clinical laboratories. Compared to broth and/or agar dilution tests as reference method, automated systems facilitate AST in a standardized setting while requiring less personnel resources and are able to provide results in shorter time courses. However, in terms of minimum inhibitory concentration (MIC) determination, these systems are regarded as inferior and thus reporting of MICs is controversial.

**Objectives:** Compare the susceptibility testing of clinically relevant gram-negative bacteria in automated AST systems of three different common manufacturers (Beckman Coulter, BD and bioMérieux) with broth microdilution tests by MERLIN or polymerase chain reaction (PCR) for molecular resistance determinants.

**Methods:** A total of 230 strains of *Enterobacterales* and 50 strains of *Pseudomonas aeruginosa* were tested for categorical agreement applying EUCAST interpretative criteria, and essential agreement (MIC  $\pm$  one 2-log level range). For *Enterobacterales*, 11 antimicrobial substances, for *P. aeruginosa* 8 substances, respectively, were compared to broth microdilution test results. Additionally, in carbapenemase producing organisms (CPO) beta-lactam susceptibility results were compared to molecular resistance determinants.

**Results:** The overall agreement for all systems with the reference method was >90%, which met the criteria of the ISO 20776-2:2007 standard. Major differences appeared in "soft criteria": hands-on time and time-to-result differed considerably among AST devices. Agreement rates depended on substance and microorganism rather than on manufacturer or method.

**Conclusion:** Automated AST results delivered MICs more rapidly than the reference method; notably, fast results did not equal less accurate results for the majority of tested substances against gram-negative pathogens.

## 012-DKMV

### Tick-borne encephalitis virus (TBEV) non-structural protein (NS1) IgG ELISA differentiating infection vs. vaccination antibody responses.

\*P. Grl<sup>1</sup>, M. Bestehorn-Willmann<sup>2</sup>, S. Zange<sup>3</sup>, J. Borde<sup>4</sup>, G. Dobler<sup>1</sup>, H. von Buttlar<sup>5</sup>

<sup>1</sup>Bundeswehr Institute of Microbiology, Virology and Rickettsiology, Munich, Germany

<sup>2</sup>University of Freiburg Medical Center and Faculty of Medicine, Division of Infectious Diseases, Freiburg, Germany

<sup>3</sup>Bundeswehr Institute of Microbiology, Central Diagnostic Department, Munich, Germany

<sup>4</sup>Health-Center Oberkirch, Oberkirch, Germany

<sup>5</sup>Bundeswehr Institute of Microbiology, Bacteriology and Toxinology, Munich, Germany

Tick-borne encephalitis (TBE) is an important central nervous system (CNS) infection in Europe and Asia caused by the tick-borne encephalitis virus (TBEV). It is a flavivirus belonging to the tick-borne group. Effective vaccines against TBE are available in the affected countries. Diagnosing TBE is challenging due to cross-reactive antibodies between different viruses of the genus *Flavivirus*, family *Flaviviridae*. Furthermore, due to the increasing vaccination rate against TBEV differentiation between infection-induced and vaccine-induced antibodies can be difficult and in many cases impossible. We present a new approach to detect antibodies against the TBEV non-structural protein 1 (NS1) as a diagnostic marker, which is exclusively indicative for virus replication in natural infection on the basis of an enzyme-

linked immunosorbent assay (ELISA). A total of 188 anonymized serum samples from the National Consultant Laboratory for TBEV were included in our study. The test was validated according to the European Laboratory Norm DIN EN ISO 15189 for diagnostic use. The enzyme-linked immunosorbent assay (ELISA) for the detection of TBEV NS1 specific IgG class antibodies reached a sensitivity of >94% and a specificity of >93% in broadly cross-reacting sera from patients with vaccinations against flaviviral diseases and single or multiple flavivirus infections. The detection of anti-NS1 antibodies is feasible and allows a reliable differentiation between different flavivirus infections and between TBEV infection versus TBE vaccination.

## 013-DKMV

False elevated minimal inhibitory concentrations for colistin due to residual detergent in re-used plastic ware

N. Joß<sup>1</sup>, M. Rhein<sup>1</sup>, N. Gold<sup>1</sup>, N. Pfennigwerth<sup>1</sup>, \*F. D. von Loewenich<sup>1</sup>

<sup>1</sup>Institute of Medical Microbiology, University of Mainz, Mainz, Germany

## Introduction

Because of the rise of multidrug-resistant Gram-negative bacteria, susceptibility test results for colistin gain increasing importance. Broth microdilution is the reference method and warnings against the use of automated systems and gradient tests due to false susceptible results exist.

## Objectives

Minimal inhibitory concentrations (MIC) for colistin measured by using commercial (E1-099-100) and customized (E1-167-100) MICRONAUT plates (Merlin, Bornheim-Hersel, Germany) were compared to test results obtained according to ISO 20776-1:2019 by the German National Reference Centre for Multidrug-resistant Gram-negative Bacteria.

## Material and Methods

63 strains of multidrug-resistant Gram-negative bacteria (57 *Enterobacterales*, 5 *Pseudomonas aeruginosa*, 1 *Acinetobacter baumannii* complex) were included. All *Enterobacterales* and *A. baumannii* complex isolates were shown to be carbapenemase producers.

## Results

65% (41/63) of colistin MIC results differed by a maximum of one two-fold dilution step. 17% (7/41) of these were clinically resistant. In 32% (20/63) the MIC measured using MICRONAUT plates was between two to seven two-fold dilution steps too high what led in 90% (18/20) to a categorical error. A thorough investigation yielded that the false-resistant results were not due to the MICRONAUT plates per se, but caused by residual detergent in re-used plastic ware.

## Conclusion

Our results favor the application of single-use plastic ware for colistin MIC testing.

## 014-DKMV

Synergism of ampicillin, gentamicin, ceftaroline and ceftriaxone against *Enterococcus faecalis* assessed *in vivo* using the *Galleria mellonella* infection model

\*L. Thieme<sup>1</sup>, A. Hartung<sup>1</sup>, O. Makarewicz<sup>1</sup>, M. W. Pletz<sup>1</sup>

<sup>1</sup>Jena University Hospital, Institute for Infectious Diseases and Infection Control, Jena, Germany

**Introduction:** *Enterococcus faecalis* is the third leading pathogen causing infective endocarditis (IE). Ongoing high patient mortality rates have prompted the emergence of novel double  $\beta$ -lactam therapies besides aminoglycoside-containing regimes. The *G. mellonella* infection model has shown increasing popularity among the non-mammalian infection models in the last years, with the main advantages being free of ethical constraints and low resource-consuming.

**Objectives:** To compare the *in vitro* and *in vivo* efficiency, we have transferred a partial checkerboard set up of ampicillin+gentamicin, ampicillin+ceftriaxone and ampicillin+ceftaroline against *E. faecalis* into the *Galleria mellonella* larvae infection model.

**Materials and methods:** Differentiation of synergistic from additive interactions was based on the evaluation of larval survival, haemolymph CFU quantity and a workgroup-internal pathology score. One-time and triple dosing schemes based on the half-life of ampicillin were applied. Pharmacokinetic data of the antibiotics in the larvae were determined via agar plate diffusion assays.

**Results:** Ampicillin and ceftriaxone synergized in a strain-dependent manner in the larvae under both dosing regimens, while the other two combinations showed additive effects. Adaptation of the dosing interval to the half-life of ampicillin led to an increased efficiency of the mono and combined antibiotics, but hampered the determination of synergistic effects. *G. mellonella* larvae allowed for a rapid, low-cost and reproducible analysis of synergistic effects of antimicrobials in an *in vivo* setting, yet not all synergistic effects observed *in vitro* could be observed in the larvae.

**Conclusion:** Our results suggest superior action of ampicillin plus ceftriaxone for the treatment of high-inoculum enterococcal infections for at least some strains, but question the benefit of the current standard gentamicin combination treatment with its high side effect profile compared to monotherapy.

## 015-DKMV

Performance of the sōna *Aspergillus* Galactomannan Lateral Flow Assay from serum samples for the diagnosis of Invasive Aspergillosis in Patients after allogeneic Hematopoietic Stem Cell Transplantation

T. Erb<sup>1</sup>, T. Held<sup>2</sup>, R. Meyer<sup>1</sup>, E. Kotter<sup>3</sup>, H. Bertz<sup>4</sup>, \*J. Held<sup>1</sup>

<sup>1</sup>Universitätsklinik Erlangen, Mikrobiologisches Institut, Erlangen, Germany

<sup>2</sup>Schön Klinik, Anästhesie & Intensivmedizin, Fürth, Germany

<sup>3</sup>University Medical Centre Freiburg, Department of Diagnostic Radiology, Freiburg, Germany

<sup>4</sup>University Medical Centre Freiburg, Department of Haematology/Oncology, Freiburg, Germany

**Question:** One cornerstone for the diagnosis of Invasive Aspergillosis (IA) is the detection of galactomannan (GM). So far, enzyme immunoassays (EIA) are used for this purpose but they are usually not performed daily resulting in an undesired delay of test results. Immunochromatographic assays, like the sōna *Aspergillus* Galactomannan Lateral Flow Assay (LFA), may overcome this disadvantage. The

LFA detects GM in serum and BALF in one hour without the need for batch testing. To compare the performance of the LFA with the Platelia™ *Aspergillus* Ag EIA (Asp-EIA) we have conducted a study on sera from patients after allogeneic hematopoietic stem cell transplantation (HSCT).

**Methods:** All patients that underwent HSCT during a one year period were enrolled. Patients with IA were classified according to the EORTC/MSG 2008 guidelines. The LFAs were analyzed with a digital reader provided by the manufacturer.

**Results:** A total of 547 sera from 101 patients after HSCT were tested. By the end of hospitalization one proven, 10 probable and 19 possible IA cases were diagnosed. The sensitivity and specificity of a single positive LFA for proven and probable IA were 80% and 57%, respectively. Repeat testing of the LFA-positive serum samples revealed that the positive result was reproducible in only 58% of samples. The sensitivity and specificity of a reproducibly positive LFA for proven and probable IA were 80% and 77%, respectively.

The Asp-EIA and the LFA are both detecting GM. To directly compare the two tests the EORTC/MSG-classification was performed with only culture and (1 $\rightarrow$ 3)- $\beta$ -D-glucan as mycological criteria. In this case one proven, 8 probable and 21 possible IA cases were diagnosed. The sensitivity and specificity of a reproducibly positive GM-test for proven and probable IA were 22% and 97% for the EIA and 78% and 70% for the LFA, respectively. The GM levels determined by both tests correlated significantly.

**Conclusions:** The LFA shows a superior sensitivity compared to the Asp-EIA. Handling of the LFA is easy and time to result is below 60 min. However, it is highly recommended to use a digital reading device and to repeat testing in case of positive results.

## 016-DKMV

Comparison of serologic biomarkers of invasive aspergillosis: evaluation of galactomannan,  $\beta$ -1,3-D-glucan, and galactomannoprotein antigen testing.

\*K. Dichtl<sup>1</sup>, U. Seybold<sup>2</sup>, H. Horns<sup>3</sup>, S. Ormanns<sup>4</sup>, J. Forster<sup>5</sup>, J. Wagener<sup>5</sup>

<sup>1</sup>Max von Pettenkofer-Institut, Lehrstuhl für Med. Mikrobiologie und Krankenhaushygiene, München, Germany

<sup>2</sup>Klinikum der Universität München, Sektion Klinische Infektiologie, München, Germany

<sup>3</sup>Klinikum der Universität München, Med. Klinik und Poliklinik 3, München, Germany

<sup>4</sup>Ludwig-Maximilians-Universität München, Institut für Pathologie, München, Germany

<sup>5</sup>Julius-Maximilians-Universität Würzburg, Institut für Hygiene und Mikrobiologie, Würzburg, Germany

Invasive aspergillosis (IA) is an invasive fungal infection caused by *Aspergillus* species and represents a major cause of morbidity and mortality in patients with impaired immunological function. A crucial determinant for survival is early diagnosis followed by targeted therapy. In this retrospective study, we evaluated the performance of two newly available serologic tests and compared their performance with the established and widely used galactomannan ELISA (GM; Bio-Rad Laboratories) representing the gold standard of serologic diagnosis.

Sera of 45 / 4 and 47 / 7 cases of proven / probable invasive aspergillosis were analyzed with the galactomannoprotein antigen ELISA (GP; Euroimmun Medizinische Labordiagnostika) and the Wako  $\beta$ -1,3-D-glucan test (BDG; Fujifilm Wako Chemicals Europe), respectively. 156 and 154

sera of patients without clinical signs for invasive aspergillosis were included as control cohorts for the respective assays. Results of GM- and GP-testing of all 423 specimens correlated strongly ( $r = 0.82$ ,  $p < 0.0001$ ). The sensitivities of both ELISAs were found to be 40 % in the proven IA cases. Including all available samples of the period of  $\pm 7$  days GM and GP seropositivity was detected in 47 % and 56 % of proven IA patients. Both ELISAs are characterized by high specificity (GM: 99 %, GP: 96 %). BDG testing was less specific (92 % vs. 99 %) but more sensitive (57 % vs. 40 %) compared to GM testing. Analysis of all sera of the  $\pm 7$  days subgroup resulted in increased per case sensitivities of 47 % (GM) and 68 % (BDG) in the proven IA population. In cases of concordant positivity (24 cases of proven / probable IA), BDG was significantly more often positive before GM (11 cases vs. one case), with a median temporal lead of nine days.

Our results demonstrate that the performance of the novel GP ELISA is similar to the Platelia GM ELISA. The BDG assay was found to be less specific. However, higher sensitivity and significantly earlier positivity of BDG testing back its usage as a diagnostic tool in cases of suspected IA. The findings underline the importance of repeated *Aspergillus* antigen testing in patients at risk for IA.

## 017-MBTV

### Heterologous production of the agrochemical pamamycin in metabolically engineered *Streptomyces albus* J1074/R2

\*J. Stegmüller<sup>1</sup>, L. Gläser<sup>1</sup>, M. Kuhl<sup>1</sup>, Y. Rebets<sup>2</sup>, C. Rückert<sup>3</sup>, J. Kalinowski<sup>3</sup>, A. Luzhetskyy<sup>2</sup>, C. Wittmann<sup>1</sup>

<sup>1</sup>Institute of Systems Biotechnology, Saarland University, Saarbrücken, Germany

<sup>2</sup>Helmholtz-Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany

<sup>3</sup>Center for Biotechnology, Bielefeld University, Bielefeld, Germany

**Introduction:** *Streptomyces*, mycelium forming soil bacteria, provide a range of industrially important natural polyketides [1]. A prominent example are pamamycins, discovered as regulators of cellular morphology and aerial mycelium formation in *S. alboniger* [2]. Recent studies revealed antibacterial activity of different pamamycin derivatives, which makes them promising for applications in the health and the agricultural industry [1].

**Objective:** In this work, the pamamycin producer *S. albus* J1074/R2 was characterized on the systems level to investigate the complex interplay between nutrient supply, morphology and production performance.

**Materials & Methods:** *S. albus* J1074/R2 was analyzed under a variety of growth conditions by metabolomics and transcriptomics to gain a detailed insight into the molecular mechanisms. Hereby, we studied the effect of medium supplements and talc microparticles to tailor the cellular morphology of the strain.

**Results:** The studies revealed a close link between morphology and pamamycin production. Upon supplementation with talc, *S. albus* J1074/R2 formed much smaller pellets and almost threefold more pamamycin, as compared to the control. This was accompanied by a global rearrangement of gene expression, including a strong activation of the pamamycin gene clusters. In addition, intracellular CoA-thioester pools were affected, which shifted the spectrum of the formed pamamycin derivatives to higher weight variants. Moreover, the nutrient status affected production, which was again reflected by global changes in

transcriptome and metabolome. The optimized set-up, provided pamamycin at a high titer of 50 mg L<sup>-1</sup>.

**Conclusion:** Pamamycin production in recombinant *S. albus* was improved. Systems biology analysis provides valuable insights for further strain improvement, using metabolic engineering.

**Acknowledgements:** This work was funded by the BMBF grant "MyBio" (FKZ 031B034A)

1. Rebets, Y., et al., *Insights into the pamamycin biosynthesis*. Angewandte Chemie, 2015. **54**(7): p. 2280-4.
2. McCann, P.A. and B.M. Pogell, *Pamamycin: a new antibiotic and stimulator of aerial mycelia formation*. The Journal of antibiotics, 1979. **32**(7): p. 673-8.

## 018-MBTV

### Construction of *in vivo* cascades for the sustainable production of monomers

\*L. Schäfer<sup>1</sup>, R. Karande<sup>1</sup>, A. Schmid<sup>1</sup>, B. Bühler<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Solar Materials, Leipzig, Germany

## Introduction

Polymers play an important role in our daily lives and have a high demand on the scale of megatons per year. The industrial production of polymer building blocks such as  $\epsilon$ -caprolactone ( $\epsilon$ -CL) is associated with critical environmental issues such as the generation of toxic waste and high energy consumption. Consequently, there is a demand for more eco-efficient and sustainable production routes.

## Objectives

The goal of our research is the development of a platform organism that is able to produce precursors for polymers such as polycaprolactone or nylon 6.

## Material and Methods

In order to develop a biotransformation of cyclohexane to these monomers, enzymes derived from the cyclohexane mineralizer *Acidovorax* sp. CHX-100 were heterologously expressed in *Pseudomonas taiwanensis* VLB120. Different plasmid-based constructs were generated and modified to prevent the accumulation of intermediate products while retaining a high catalytic activity.

## Results

A biocatalytic cascade based on a cytochrome P450 monooxygenase (CYP), a cyclohexanol dehydrogenase (CDH), a Baeyer-Villiger-monooxygenase (BVMO) was constructed for  $\epsilon$ -CL synthesis from cyclohexane and optionally was amended by a lactonase to end up with the hydrolyzed precursor 6-hydroxyhexanoic acid (6OH-HA). The construction of an expression plasmid with one operon harboring the first three enzymes resulted in a decent activity of 37 U g<sub>CDW</sub><sup>-1</sup>. However, this activity was not stable as cyclohexanol, which inhibits the BVMO accumulated with time. The introduction of a second promoter could completely circumvent this problem. Intrinsic hydrolases of *P. taiwanensis* constituted another issue so that both  $\epsilon$ -CL and 6OH-HA accumulated as products. The introduction of a fourth enzyme, a highly active lactonase, could completely



push the cascade towards 6-OH HA with a high activity of 45 U g<sub>CDW</sub><sup>-1</sup>.

## Conclusions

The design of biocatalytic cascades requires knowledge on the *in vivo* (co-)operation of the involved enzymes, which is a prerequisite to rationally engineer a streamlined reaction. We could show that *P. taiwanensis* can be used as a platform organism to efficiently synthesize monomers.

### 019-MBTv

Metabolic Engineering of *Corynebacterium glutamicum* for Production of UDP-N-acetylglucosamine

\*C. Desiderato<sup>1</sup>, R. Gauttam<sup>1</sup>, D. Radoš<sup>2</sup>, A. Schwentner<sup>1</sup>, G. M. Seibold<sup>1</sup>, H. Link<sup>2</sup>, B. Eikmanns<sup>1</sup>

<sup>1</sup>Ulm University, Institute of Microbiology and Biotechnology, Ulm, Germany

<sup>2</sup>Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Uridine diphosphate-N-acetylglucosamine (UDP-GlcNAc) is an acetylated aminosugar nucleotide which serves as precursor in bacterial cell wall synthesis and is involved in glycosylation reactions. Nucleotide sugars are currently either produced chemically or *in vitro* by enzyme cascades. However, *in vitro* synthesis requires costly substrates and in most cases purified enzymes. An alternative way would be the microbial production of nucleotide sugars from cheap substrates. In this study, we aim to engineer the non-pathogenic, Gram-positive soil bacterium *Corynebacterium glutamicum* as host for UDP-GlcNAc production.

To increase UDP-GlcNAc formation, the native *glmS*, *glmU*, and *glmM* genes and *glmM* of *E. coli*, encoding the enzymes for UDP-GlcNAc synthesis from fructose-6-phosphate, were overexpressed in different combinations and on different plasmids in *C. glutamicum* GRS43 [1], which lacks among others the *nagB* gene for glucosamine degradation. The recombinant strains were cultivated in CGXII minimal medium supplemented with glucose as sole carbon source. Expression of the respective genes was examined by activity assays or Western blot analysis. UDP-GlcNAc concentrations were determined by LC-MS/MS analysis.

While the reference strain *C. glutamicum* GlcNCg1 with an empty plasmid in the exponential growth phase contained intracellularly only about 0.25 mM UDP-GlcNAc, the best engineered strain GlcNCg4 accumulated around 14 mM UDP-GlcNAc. The extracellular concentrations in the exponential growth phase did not exceed 2 mg/L. In the stationary phase, about 60 mg UDP-GlcNAc/L were observed extracellularly with strain GlcNCg4, indicating the potential of *C. glutamicum* to produce the activated sugar.

We here show UDP-GlcNAc production from glucose with *C. glutamicum* and its release into the culture medium. To our knowledge, the observed levels are the highest obtained with microbial hosts, emphasizing the potential of *C. glutamicum* as suitable platform for activated sugar production.

[1] Unthan *et al.*, "Chassis organism from *Corynebacterium glutamicum* - a top-down approach to identify and delete irrelevant gene clusters," *Biotechnol. J.*, vol. 10, no. 2, pp. 290–301, 2015.

### 020-MBTv

Bromination of L-tryptophan in a fermentative process with *Corynebacterium glutamicum*

\*K. H. Veldmann<sup>1</sup>, S. Dachwitz<sup>2</sup>, J. M. Risse<sup>3</sup>, J. H. Lee<sup>4</sup>, N. Sewald<sup>2</sup>, V. F. Wendisch<sup>1</sup>

<sup>1</sup>University Bielefeld, Genetics of Prokaryotes, Bielefeld, Germany

<sup>2</sup>University Bielefeld, Organic and Bioorganic Chemistry, Bielefeld, Germany

<sup>3</sup>University Bielefeld, Fermentation Technology, Bielefeld, Germany

<sup>4</sup>Kyungshung University, Major in Food Science & Biotechnology, Busan, South Korea

Brominated compounds such as 7-bromo-L-tryptophan (7-Br-Trp) occur in Nature. Many synthetic and natural brominated compounds have applications in the agriculture, food and pharmaceutical industries, for example, the 20S-proteasome inhibitor TMC-95A that may be derived from 7-Br-Trp [1]. Mild halogenation by cross-linked enzyme aggregates containing FADH-dependent halogenase, NADH-dependent flavin reductase, and alcohol dehydrogenase [2] as well as by fermentation with recombinant *Corynebacterium glutamicum* expressing the genes for the FADH-dependent halogenase RebH and the NADH-dependent flavin reductase RebF from *Lechevalieria aerocolonigenes* [3] have recently been developed as green alternatives to more hazardous chemical routes. In this study, the fermentative production of 7-Br-Trp was established. The fermentative process employs an L-tryptophan producing *C. glutamicum* strain [4] expressing *rebH* and *rebF* from *L. aerocolonigenes* for halogenation and is based on glucose, ammonium and sodium bromide. *C. glutamicum* tolerated high sodium bromide concentrations, but its growth rate was reduced to half-maximal at 0.09 g L<sup>-1</sup> 7-Br-Trp. This may be, at least in part, due to inhibition of anthranilate phosphoribosyltransferase by 7-Br-Trp since anthranilate phosphoribosyltransferase activity in crude extracts was half-maximal at about 0.03 g L<sup>-1</sup> 7-Br-Trp. Fermentative production of 7-Br-Trp by recombinant *C. glutamicum* was scaled up to a working volume of 2 L and operated in batch and fed-batch mode. The titers were increased from batch fermentation in CGXII minimal medium with 0.3 g L<sup>-1</sup> 7-Br-Trp to fed-batch fermentation in HSG complex medium, where up to 1.2 g L<sup>-1</sup> 7-Br-Trp were obtained. The product isolated from the culture broth was characterized by NMR and LC-MS and shown to be 7-Br-Trp.

[1] Koguchi Y., et al. (2000) *J. Antibiot.* 53:105-109

[2] Frese M., et al. (2015) *Angew. Chem. Int. Ed.* 54:298-301

[3] Veldmann K.H. (2019) *J. Biotechnol.* 291, 7-16

[4] Purwanto H.S. (2018) *J. Biotechnol.* 282:92-100

### 021-MBTv

Valorization of lignin-derived aromatics using metabolically engineered *Corynebacterium glutamicum*

\*J. Becker<sup>1</sup>, M. Kuhl<sup>1</sup>, M. Kohlstedt<sup>1</sup>, C. Wittmann<sup>1</sup>

<sup>1</sup>Universität des Saarlandes, Institut für Systembiotechnologie, Saarbrücken, Germany

Introduction: As integral constituent of terrestrial plants, lignin is the second most abundant polymer on earth. Every year, 140 million tons of lignin accumulate as waste product by the plant processing industry and are mainly burned due to the lack of alternative valorization routes [1]. Lignin is hence the most underutilized biobased renewable today.

Objectives: *Corynebacterium glutamicum* is a very well-known industrial producer [2] and is able to degrade a large spectrum of aromatic compounds [3]. Here, *C. glutamicum*

should be engineered to convert aromatics into *cis,cis*-muconate (MA), a precursor of substantial value to derive commercial chemicals such as adipate and terephthalate [3].

**Materials & Methods:** The  $\beta$ -ketoadipate pathway for aromatic catabolism was disrupted downstream of MA by deletion of *catB*, encoding muconate cycloisomerase, and the resulting strain was cultivated with glucose as growth substrate and different aromatics as biotransformation substrates.

**Results:** The strain was able to convert different aromatic compounds into MA with a molar yield of 100%. The production efficiency was substantially improved by overexpression of *catA*, encoding catechol 1,2-dioxygenase, the key enzyme forming MA. In a fed-batch process, the optimized MA-2 strain accumulated 85 g L<sup>-1</sup> MA from catechol with a volumetric productivity of 2.4 g L<sup>-1</sup> h<sup>-1</sup> [3]. The strain was also used to produce MA from depolymerized softwood lignin.

**Conclusion:** Our findings open the door to valorize lignin by metabolic engineering of *C. glutamicum*.

**Acknowledgement:** This project was funded by the BMBF through the grants "Bio2Nylon" (No. 03V0757) and "LignoValue" (No. 01DN17036).

#### References:

[1] Becker & Wittmann, 2019. Biotechnol. Adv. 37, 107360.

[2] Becker et al., 2018. Metab. Eng. 50, 122-141.

[3] Shen et al., 2012. Appl. Microbiol. Biotechnol. 95, 77-89.

[4] Becker et al., 2018. Microb. Cell Fact. 17, 115.

---

#### 022-MBTV

The author has not agreed to a publication.

---

#### 023-MBTV

Design of a H<sub>2</sub>-driven enzymatic cascade for the synthesis of methylated *N*-heterocycles

A. Al-Shameri<sup>1</sup>, N. Borlinghaus<sup>2</sup>, L. Weinmann<sup>2</sup>, P. Scheller<sup>2</sup>, B. Nestl<sup>2</sup>, \*L. Lauterbach<sup>1</sup>

<sup>1</sup>Technische Universität Berlin, Institut für Chemie, Berlin, Germany

<sup>2</sup>Universität Stuttgart, Institute of Biochemistry and Technical Biochemistry, Stuttgart, Germany

#### Introduction:

*N*-heterocycles are valuable precursors for the synthesis of various pharmaceuticals [1]. Up to date, only a few chemical and biological strategies exist for the selective synthesis of saturated *N*-heterocycles.

#### Objectives:

Our aims are the design of a synthetic enzymatic cascade for the H<sub>2</sub>-driven production of methylated *N*-heterocycles and understanding of the interplay between the individual oxidoreductases.

#### Materials & methods:

We designed an enzymatic cascade to synthesis methylated *N*-heterocycles consists of an engineered putrescine oxidase (PuO<sup>E203G</sup>) [2], an imine reductase (IRE<sup>D</sup>) [3] and a NADP<sup>+</sup>-reducing hydrogenase derivative (SH) from *Ralstonia eutropha* [4]. The O<sub>2</sub>-dependent PuO<sup>E203G</sup> oxidizes diamines to the corresponding imines, which are subsequently reduced by the NADPH-dependent IRE<sup>D</sup> to the saturated *N*-heterocycles. The O<sub>2</sub>-tolerant SH derivative catalyses the H<sub>2</sub>-driven recycling of NADPH [4].

#### Results:

Substituted pyrrolidines and piperidines were obtained with up to 97% product formation in a one-pot reaction directly from the corresponding diamine substrates. The formation of up to 93% ee gave insights into the specificity and selectivity of the putrescine oxidase. The H<sub>2</sub>-driven NADPH regeneration by the SH derivative was well sufficient and surpassed the commonly used glucose-6-phosphate dehydrogenase system with no by-product formation and 100% atom efficiency [2].

#### Conclusion:

In conclusion, we have developed an enzymatic cascade reaction, allowing access to pyrrolidine and piperidine heterocycles from substituted diamines. The described enzymatic cascade avoids the consumption of any organic solvents or toxic compounds demonstrating the sustainability and greenness of this approach.

#### References:

- [1] Vitaku E, D.T. Smith, J.T. Njardarson (2014), J Med Chem. 26, 10257-74
- [2] Al-Shameri, N. Borlinghaus, L. Weinmann, P. Scheller, B. Nestl, L. Lauterbach, (2019), Green Chem, 21, 1396–1400.
- [3] N. Scheller and B. M. Nestl, (2016), Appl Microbiol Biotechnol, 100, 10509–10520.
- [4] Preissler, H.A. Reeve, T. Zhu, K. Urata, L. Lauterbach, L.L. Wong, K.A. Vincent, O. Lenz, (submitted)

---

#### 024-MBTV

##### Comparative evaluation of expression modules for recombinant biosurfactant-production by *Pseudomonas putida*

S. Kubicki<sup>1</sup>, I. Bator<sup>2</sup>, R. Weihmann<sup>1</sup>, S. Linden<sup>1</sup>, A. Loeschcke<sup>1</sup>, T. Drepper<sup>1</sup>, T. Tiso<sup>2</sup>, L. M. Blank<sup>2</sup>, K. E. Jaeger<sup>1,3</sup>, \*S. Thies<sup>1</sup>

<sup>1</sup>Heinrich-Heine-University Düsseldorf, Institute of Molecular

Enzyme Technology, Juelich, Germany

<sup>2</sup>RWTH Aachen University, Institute of Applied Microbiology iAMB, Aachen, Germany

<sup>3</sup>Forschungszentrum Jülich, Institute of Bio- and Geosciences IBG 1: Biotechnology, Jülich, Germany

The construction of recombinant strains is an important strategy for the production of diverse natural products and thus represents a key element of a biobased economy. To this end, sufficient expression levels of biosynthetic gene clusters have to be achieved. *Pseudomonas putida* has emerged as a promising platform in this context with several functional expression tools available. However, comparative studies are largely lacking. Here, we compared different inducible expression systems in *P. putida* KT2440 with rhamnolipids as target compounds which are valuable biosurfactants with potential applications in cosmetics, agriculture, food, and pharmaceuticals.

We have used an efficient system for transfer and expression of biosynthetic modules named *yTREX<sup>Tn7</sup>* to establish the respective biosynthetic pathway within the host. This system features site-specific chromosomal integration to allow a comparative evaluation of different modules in terms of expression strength by measuring fluorescence and product accumulation as determined by colorimetry and HPLC.

The modular architecture of *yTREX<sup>Tn7</sup>* allows the straightforward exchange of bricks like cargo modules and regulatory elements. The cargo modules applied here contain rhamnolipid biosynthesis genes obtained from various organisms in transcriptional fusion with an expression reporter. In total, 16 stable production strains were constructed by implementation of different modules. Highest product titers and reporter outputs were obtained using a salicylate-responsive expression module with a yield of up to 1 g/L of *P. aeruginosa* type monorhamnolipids after 24 h at lab-scale cultivation. This was in line with the, among all strains, fastest increase of the reporter signal after induction. The optimal inductor concentration was determined to be 2 mM. We also developed a novel colorimetric assay for a fast assessment of rhamnolipid levels.

In summary, we identified efficient biosynthesis modules for the production of rhamnolipids with a modular construction of the expression cassette allowing for the addition of further bricks to enhance the productivity, such as encode genes needed for precursor supply.

---

## 025-GIMPV

### Identification of antibiotics that do not cause Shiga-toxin mediated renal disease in *C. rodentium* $\phi$ stx2dact-infected mice

\*S. Mühlen<sup>1,2</sup>, I. Ramming<sup>2</sup>, M. Pils<sup>1,2,3</sup>, M. Koepfel<sup>4</sup>, B. Stecher<sup>4</sup>, P. Dersch<sup>1,2</sup>

<sup>1</sup>University of Münster, Department of Infectiology, Münster, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Molecular Infection Biology, Braunschweig, Germany

<sup>3</sup>Helmholtz Centre for Infection Research, Mouse Pathology, Braunschweig, Germany

<sup>4</sup>Max-von-Pettenkofer Institut, München, Germany

Enterohaemorrhagic *Escherichia coli* (EHEC) is a food-borne pathogen that causes disease ranging from watery diarrhoea to acute renal failure. Disease progression upon infection is attributed to the production and release of Shiga toxins (Stx). The treatment of EHEC infections is currently supportive and the use of antibiotics is highly controversial due to reports that antibiotics induce Shiga toxin expression and may, therefore, increase the risk of HUS development. While Stx-induction has been assessed *in vitro* with a plethora of antibiotics and a variety of EHEC strains, *in vivo* confirmation has been hampered by the lack of an adequate mouse model. In 2012, Mallick *et al.* introduced the lysogenic phage encoding *stx2d* from EHEC into the mouse pathogen *C. rodentium*, creating a strain that mimics a human STEC infection in mice.

Here, we assessed a number of commercially available antibiotics for their ability to induce the production and release of Shiga toxin *in vitro* and *in vivo*. We infected mice with *C. rodentium*  $\phi$ stx2d and determined whether any antibiotics could clear the infection without detrimental effects to the host. Using the fluoroquinolone antibiotic enrofloxacin as an example for a known Shiga toxin-inducing antibiotic, we observed that the mice showed rapid weight loss after treatment-onset even though the infection was

cleared. Kidney histology and serology for the kidney injury marker BUN (blood urea nitrogen) showed kidney damage comparable to that observed for infected but untreated mice. A number of tested antibiotics were, however, able to clear the infection without resulting in kidney damage. Interestingly, rifampicin, a protein synthesis inhibitor, was unable to clear infection at the concentrations used in this study. Rifampicin did not, however, induce Stx-mediated kidney damage, supporting *in vitro* data suggesting that rifampicin inhibits Shiga toxin production.

This data suggests that while some antibiotics induce the SOS response and increase Stx production and release, other antibiotics may well be used as treatment options, especially during outbreaks.

<sup>1</sup>Mallick EM *et al.* (2012). *J Clin Invest.* 2012 Nov;122(11).

---

## 026-GIMPV

withdrawn

---

## 027-GIMPV

The fate of phytate in the gut: *myo*-inositol degradation by *Salmonella* Typhimurium

\*T. M. Fuchs<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institut für Molekulare Pathogenese, Jena, Germany

*Myo*-inositol (MI) is a polyol abundant in soil. Its phosphorylated form, inositol hexakisphosphate or phytate, serves as a phosphorus storage in plants, especially in seeds. *Salmonella* Typhimurium (*S. Typhimurium*) strains are able to grow with MI as the sole carbon and energy source (1). The 22.6-kb genomic island responsible for this metabolic capacity comprises 19 protein-encoding *iol* genes and two sRNA genes. An extended lag phase of 60 hours and a reversible bistable phenotype are unique features of *S. Typhimurium* growth in the presence of MI (2). On the single cell level, flow cytometry analysis based on promoter::*gfp*-fusions revealed that in the presence of MI, a small subpopulation switches its *iol* genes from the "OFF" to the "ON" state at the end of the lag phase. This heterogeneity is abolished in the presence of at least 0.55% hydrogen carbonate that might act as an *in vivo* stimulus. Oral infection experiments with cattle, pigs and chicken demonstrated a significant contribution of MI utilization to a *S. Typhimurium* infection. A set of factors was identified that helps the enteropathogen to orchestrate the complex regulatory architecture of MI utilization when it encounters varying substrate conditions as in the gut. The global silencer H-NS, the main repressor *IolR* and the catabolite regulator protein CRP are antagonized by the orphan activator *ReiD* (3), the virulence regulator *SsrB* and the novel regulatory sRNA *RssR* (4). Moreover, the high binding affinity of *IolR* to its target promoters avoids untimely and costly expression of this catabolic pathway (5). Taken together, the investigation of MI degradation by salmonellae provides insights into the molecular mechanisms by which a pathogen acquires, integrates, and utilizes a novel metabolic property.

## References:

1. Kröger C & Fuchs TM (2009). *J Bacteriol* 191: 545-554.

2. Kröger C, Srikumar S, Ellwart J, Fuchs TM (2011). *J Bacteriol* 193: 1427-1435.

3.Rothhardt JE, Kröger C, Broadley SP, Fuchs TM (2014). Mol Microbiol 94: 700-712.

4.Kröger C, Hellinckx J, Fuchs TM (2018). Sci Rep 8:17739.

5.Hellinckx J, Heermann R, Felsl A, Fuchs TM (2017). Sci Rep 7:44362.

---

## 028-GIMPV

### Global discovery of small regulatory RNAs in *Clostridium difficile*

M. Fuchs<sup>1</sup>, V. Lamm<sup>1</sup>, \*F. Faber<sup>1</sup>

<sup>1</sup>Institute of Molecular Infection Biology, Würzburg, Germany

Small non-coding RNAs (sRNAs) are important modulators of post-transcriptional gene regulation in a broad range of bacterial species which regulate both physiological and virulence pathways. The discovery of these post-transcriptional regulatory networks has added a new layer of complexity to gene regulation in bacteria. It is now well established that non-coding RNA regulators modulate mRNA translation and stability in a wide range of bacteria, however, in *C. difficile* relevant RNA-binding proteins and central sRNAs are still unexplored.

We have combined dRNA-seq based annotation of sRNA candidates with density-gradient centrifugation of native cell lysates coupled to RNA-sequencing and LC-MS/MS of gradient fractions (Grad-seq) to identify functional non-coding transcripts and predict their associated RNA-binding proteins in *C. difficile*.

Our analyses have identified a spectrum of sRNA players that associate with the global RBP Hfq and other novel RNA-binding protein candidates. While Hfq functions have been intensively studied in many gram-negative bacteria, its role in gram-positive bacteria, and especially in *C. difficile*, remains elusive.

We conclude, our discovery of a wide spectrum of noncoding RNA molecules that associate with different RNA-binding proteins suggests the existence of a rich layer of post-transcriptional control in *C. difficile*.

---

## 029-VPV

Isolation of therapeutic bacteriophages against carbapenem-resistant *Acinetobacter baumannii*

\*A. Madi<sup>1</sup>, F. Stachon<sup>1</sup>, S. Schubert<sup>1</sup>, G. Maschkowitz<sup>1</sup>, H. Fickenscher<sup>1</sup>

<sup>1</sup>Institute for Infection Medicine, Kiel, Germany

*Acinetobacter* (*A.*) *baumannii* is a human pathogen with pronounced intrinsic and acquired antibiotic resistance, with a high degree of tenacity and it is a dreaded cause of nosocomial infections. *A. baumannii* strains with carbapenem resistance can cause fatal outbreaks in intensive care units. Often, the antibiotic drug colistin is one of the last therapeutic options, with considerable side effects. Therapeutic bacteriophages form an alternative valuable antibacterial therapeutic option.

Initially, we isolated five bacteriophages from wastewater using a carbapenem-sensitive outbreak strain of *A. baumannii*. When tested against clinical *A. baumannii* strains, the bacteriophages showed a narrow host range and no activity against the carbapenem-resistant outbreak strain. Isolation using wastewater from sewage plants in Kiel,

Hamburg and Berlin and the carbapenem-resistant *A. baumannii* outbreak isolate yielded five additional bacteriophages. Although these bacteriophages were able to lyse nearly all host cells, the carbapenem-resistant *A. baumannii* strain developed some resistant colonies. Bacteriophages isolated against these previously resistant bacteria were unable to lyse the original host strains.

Although the spectrum of bacteriophages against *A. baumannii* seems to be narrowly restricted, the isolation of phages seems feasible which specifically target multidrug-resistant isolates. Phage cocktails might be able to prevent the emergence of multidrug-resistant strains, especially after further modification of the host specificity of already isolated bacteriophages.

---

## 030-VPV

Biology and genetics of phages from German waste water treatment plants of livestock slaughterhouses and their potential to combat multidrug-resistant *P. aeruginosa* isolates

S. Schnehle<sup>1</sup>, C. Jäckel<sup>1</sup>, M. Savin<sup>2</sup>, S. Schmoger<sup>1</sup>, A. Käsbohrer<sup>1,3</sup>, M. Borowiak<sup>1</sup>, J. Kreyenschmidt<sup>2</sup>, \*J. A. Hammer<sup>1</sup>

<sup>1</sup>Bundesinstitut für Risikobewertung, Biological Safety, Berlin, Germany

<sup>2</sup>University of Bonn, Faculty of Agriculture, Institute of Animal Sciences, Bonn, Germany

<sup>3</sup>Institute for Veterinary Public Health, University of Veterinary Medicine, Wien, Austria

**Questions:** Pseudomonads are Gram-negative bacteria of the Pseudomonadaceae for which currently 191 valid species have been reported. The species *P. aeruginosa* has a high impact in clinical settings, as they efficiently exchange DNA (i.e. resistance plasmids) with other bacteria. Antimicrobial resistance of multidrug-resistant isolates is multifactorial and based on chromosomal and extrachromosomal factors. Due to the steadily increasing number of multidrug-resistant *Pseudomonas* isolates in clinical settings, efficient strategies for control of these bacteria are needed. As bacteriophages have been reported as suitable alternatives for the treatment of multidrug-resistant isolates, this study aimed to determine the occurrence of lytic phages from waste water treatment plants of poultry and pig slaughterhouses.

**Methods:** The recovered phages were characterized in their phenotypic (host range, plaque formation, TEM) and genotypic properties (WGS) to determine their potential for the treatment of *Pseudomonas* spp. bacteria.

**Results:** In general, all recovered phages exhibited a highly restricted host range, which is limited to the species *P. aeruginosa*. However, the spectrum and the number of lysed isolates vary substantially, and provide thus a good basis for the development of a phage cocktail with broad activity. Overall, the phages were shown to be stable under a wide range of pH and temperature conditions. On the basis of their virion morphology, the phages were allocated to different groups of *Pseudomonas* phages. WGS and bioinformatics analyses revealed detailed information on their phylogenetic relationship to different virus-genera. Furthermore, sequence analyses indicated that the recovered phages are suitable for therapeutic issues as they did not carry antimicrobial resistance and/or virulence determinants.

**Conclusions:** On the basis of the prevailing results, lytic phages can be used to combat *P. aeruginosa* with high efficacy. However, in order to develop a broad active and highly efficient phage compound that prevents development

of phage resistant bacterial isolates, the cocktails need to be carefully composed.

### 031-VPV

Go with the Flow: Quantifying the Waterborne Transport of Phages in a Soil Matrix

\*X. You<sup>1</sup>, R. Kallies<sup>1</sup>, K. Hild<sup>1</sup>, A. Hildebrandt<sup>2,3</sup>, H. Harms<sup>1</sup>, A. Chatzinotas<sup>4</sup>, L. Y. Wick<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Computational Hydrosystems, Leipzig, Germany

<sup>3</sup>Friedrich Schiller University Jena, Institute of Geoscience, Jena, Germany

<sup>4</sup>German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

#### Background:

Phages (*i.e.* viruses that infect bacteria) have been considered as good tracers for the hydrological transport of colloids and (pathogenic) viruses. They are typically quantified as total virus particles or plaque forming units (PFU) of infectious phages. Little, however, is known about the transport of phages in soil as interactions with heterogeneous soil matrices may hamper their transport and infectivity, and thus have effects on soil microbial ecosystem functioning.

#### Objectives:

Here we quantified waterborne transport of phages in laboratory soil columns and evaluated its effects on the specific infectivity of phages. The specific infectivity is defined by the ratio of total particle to PFU and is a measure for the minimum particle numbers needed to create a single infection.

#### Methods:

Three lytic phages of different morphology and physicochemical surface properties (*i.e.* hydrophobic *E. coli* phage T4 and the hydrophilic marine *Pseudoalteromonas* phage HS2 and HM1) were applied in saturated laboratory soil columns. Breakthrough of infective and total phages was quantified by PFU and quantitative PCR (qPCR) in the column effluents and compared to breakthrough of non-reactive bromide and chloride tracers.

#### Results:

We found significant breakthrough of all phages in soil filled saturated columns. Infective phages were retained in a descending order of HM1 (61%), HS2 (30%) and T4 (24%). qPCR quantification however revealed even more significant retention of total particles for all phages and pointed at a clear reduction of the specific infectivity relative to the influents. All phages showed faster breakthrough through soil than non-reactive tracers.

#### Conclusions:

Our data suggest that phages are efficiently retained by soil matrices with retention extents depending on the phage properties. Transport decreased the specific infectivity of the phages (*i.e.* increased relative fractions of infective phages) in the effluents pointing at an increased infection hazard for host bacteria. Due to their absence in terrestrial systems and

their high PFU-based recoveries, marine phages can be considered as good tracers to assess viral transport in soil.

### 032-VPV

The viral community along the Holtemme River

\*R. Kallies<sup>1</sup>, G. Christian<sup>2</sup>, A. Chatzinotas<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>University of Vienna, Department of Limnology and Bio-Oceanography, Vienna, Austria

#### 1. Introduction

Rivers carry water and nutrients, act as drainage channels for surface water and serve as habitat and food source for many earth's organisms. Due to the increasing pressure from urbanisation and intensified agriculture, many rivers have been changing; stressors acting alone or simultaneously. These changes influence population dynamics and the diversity of river organisms, among them also microorganisms. Viruses are one key factor for microorganism mortality, drive diversification and evolution through gene transfer and are key contributors to the mineralization of nutrients.

#### 2. Objectives

The objectives of this study were to: 1) identify viruses present along a 40 km Holtemme river stretch and 2) determine environmental factors that may influence virus community structure.

#### 3. Materials & methods

Thirteen sampling sites were selected along the Holtemme River, a 48 km long river that starts in the Harz Mountains, Germany. Sampling sites were chosen to reflect different influences, such as natural environment, waste water treatment plants (WWTP), factories, agriculture and restored waters. Water was collected to count virus particle abundancies using flow cytometry and to extract DNA for viral metagenome analysis. Sequencing libraries were prepared from both, random amplified DNA and using the NEBNext Ultra II FS technology.

#### 4. Results

The number of virus particles increased over the course of the river reaching peaks at WWTP introductions. Viral communities were dominated by novel sequences and members of the *Caudovirales* order and *Microviridae* family followed by insect- and plant-specific viral sequences. Highest diversity of viral sequences was found in urban areas. Viral sequence communities clustered according to their origin from natural, urban or agricultural sites demonstrating the anthropogenic impact on viral communities in this river.

#### 5. Conclusion

Our results provide potentially valuable data for assessing spatial dynamics of river viral communities in the context of anthropogenic stress and will further help to understand how environmental factors affect virus communities in this river system.



### 033-VPV

Genome-informed microscopy reveals viral infections of uncultivated archaea in a terrestrial subsurface aquifer

\*V. Turzynski<sup>1</sup>, J. Rahlff<sup>1</sup>, C. Moraru<sup>2</sup>, A. J. Probst<sup>1</sup>

<sup>1</sup>University Duisburg Essen, Institute for Environmental Microbiology and Biotechnology - Aquatic Microbial Ecology, Essen, Germany

<sup>2</sup>Carl von Ossietzky University Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany

**Introduction:** Candidatus *Altiarchaeum hamiconexum* forms nearly pure biofilms in terrestrial subsurface aquifers (up to 95% of cells) and has an autotrophic metabolism, thus heavily impacts carbon cycling in these environments. Microbial interactions of Ca. *A. hamiconexum* with bacteria and symbiosis with another uncultivated archaeon are known, yet archaeal viruses infecting Ca. *A. hamiconexum* and their subsequent impact on the ecosystem's food web remain uncertain.

**Objectives:** Here, we show that Ca. *A. hamiconexum* is a frequent target of a previously unknown putative archaeal virus.

**Material & Methods:** Putative viral infections on Ca. *A. hamiconexum* cells were analysed by genome-informed microscopy, based on a modified fluorescence *in situ* hybridization method (direct-geneFISH a.k.a. viral FISH). Probes for direct-geneFISH were specifically designed to target a putative 8.9 kb circular viral genome, which was previously *in silico* identified as a putative virus. Key findings were obtained via manual enumeration of viral infections, classified into three infection categories.

**Results:** By using genome-informed microscopy, viral infections of Ca. *A. hamiconexum* cells showed lytic features that could have implications for the integrity of the archaeal host and thus on the entire carbon cycle in the ecosystem. 523 infections of 18,411 counted archaeal cells revealed a low percentage of virus adsorption (7.6%), few advanced infections (17.4%), and many states of cell lysis (75%).

**Conclusion:** Overall, we conclude that Ca. *A. hamiconexum*'s viruses have major implications in terms of controlling host abundance, microbial community composition, biogeochemical cycling and nutrient fluxes in subsurface aquifers.

### 034-VPV

**Environmental risks of nanomaterials through prophage activation: what we can learn from the metabolic heat evolution.**

\*T. Maskow<sup>1</sup>, J. Xu<sup>2</sup>, F. L. Jiang<sup>2</sup>, Y. Liu<sup>2</sup>, B. Kiesel<sup>1</sup>, R. Kallies<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Wuhan University, College of Chemistry and Molecule Sciences, Wuhan, China

**Question:** A few thousands of tons of nanoparticles and quantum dots (QDs) are yearly produced worldwide and significant parts are released into ecosystems. An important ecotoxicological aspect has been largely overlooked. It is the activation of silent bacteria by the released nanomaterial. Activated viruses once released catalyze an autocatalytic process with a potentially exponentially killing rate of bacteria. Is it possible to shed light on this underestimated process by recording the released metabolic heat in real time and interpreting the results thermodynamically and kinetically?

**Methods:** To answer the question *Escherichia coli* with (λ+) and without (λ-) lambda prophages were treated with test chemicals and QDs in different concentrations. The process of prophage activation and bacteria killing were monitored in real time as heat production rate and by off-line measurements. The off-line measurements include the number of activated phage particles and surviving *E. coli* cells, the intracellular levels of ROS, superoxide dismutase activity and lipid peroxides. The influence of protective agents (radical catchers) and metal-ion chelators were investigated. Mathematical simulations were used to generate a coherent image of the prophage activation from this data.

**Results and Conclusion:** Calorimetry allowed the real time monitoring of the prophage activation and thus qualify itself as a future screening tool for testing chemicals concerning their ability to activate prophages. CdTe QDs as an example activate prophages at already nanomolar concentrations range. The activation is mainly triggered by DNA damages due to oxidative stress induced by the CdTe QDs. Freely dissociated Cd<sup>2+</sup> contributes depending on the functionalization of QDs between 15 and 25 % on the prophage activation. This paper will deepen the understanding of effects of QDs on microbial environments and provide new concerns for the potential environmental risks of quantum dots.

### 035-VPV

**Microviscosity control in *Pantoea stewartii* biofilms by an intrinsic exopolysaccharide modifying enzyme and by bacteriophage tailspike proteins**

T. Imscher<sup>1</sup>, Y. Roske<sup>2</sup>, V. Dunsing<sup>3</sup>, A. Singhal<sup>4</sup>, A. Grafmüller<sup>4</sup>, U. Heinemann<sup>1</sup>, S. Chiantia<sup>3</sup>, \*S. Barbirz<sup>1</sup>

<sup>1</sup>Universität Potsdam, Physikalische Biochemie, Potsdam, Germany

<sup>2</sup>Max-Delbrück-Centrum für Molekulare Medizin, Berlin, Germany

<sup>3</sup>Universität Potsdam, Physikalische Zellbiochemie, Potsdam, Germany

<sup>4</sup>Max-Planck-Institut für Kolloid- und Grenzflächenforschung, Potsdam, Germany

The properties of bacterial biofilms are dictated by the macromolecules that build up a complex matrix to control embedding of cells, access of metabolites and defense against antimicrobials and bacteriophages. Whereas highly stable protein structures like curli or insoluble polysaccharides like cellulose may provide rigid mechanical scaffolds, biofilms may also be composed from highly soluble anionic exopolysaccharide (EPS) as the main constituent. These biofilms are functional as protective barriers due to their high viscosity.

We have analyzed the matrix properties of stewartan, a mega-dalton sized anionic polysaccharide and major EPS biofilm component of the plant pathogen *Pantoea* (*P.*) *stewartii* [1]. Viscosity of the stewartan matrix can be controlled by different glycan-modifying enzymes. WceF is a putative biofilm remodeling protein encoded on a *P. stewartii* biofilm synthesis gene cluster [2]. Crystal structure analysis revealed that WceF has striking structural similarity to bacteriophage tailspike (TSP) hydrolases that modify long polysaccharide chains, with a typical trimeric β-helix fold. WceF hydrolyzes stewartan, but with notably lower efficiency than the stewartan-targeting TSP from bacteriophage φEa1h. These differences illustrate that stewartan is not a fully inert meshwork. Rather, fluorescence correlation spectroscopy showed that the mobility of particles in stewartan depends on their size and surface chemistry. The stewartan matrix acts as a molecular sieve with properties depending on ionic strength and divalent cations that is effective in trapping of bacteriophages. Coarse-grained

computational simulations emphasize that mesh size is an inherent property of stewartan determined by its the glycan composition and regulated by polymer chain entanglement. Our results stress that major physicochemical properties of the biofilm can be controlled by glycan-active enzymes from both a host bacterium and a bacteriophage.

[1] Dunsing, V., Irmscher, T., Barbirz, S., and Chiantia, S. (2019) *Biomacromolecules* 20: 3842-3854.

[2] Carlier, A., Burbank, L., and von Bodman, S.B. (2009) *Mol. Micro.* 74: 903-913.

---

### 036-VPV

#### Phage - prophage association network: Inferring functional similarity on large scale

\*O. Popa<sup>1</sup>

<sup>1</sup>Heinrich-Heine Universität, Institute of quantitative and theoretical biology, Düsseldorf, Germany

Bacteriophages, also known as phages, are viruses of bacteria and archaea. Their impact on prokaryotic evolution has been investigated for long time but until today fundamental mechanism are not fully understood. The genetic relation of phages and prophages with their hosts and among each other is barely investigated on a large scale. In this study we analysed 4,088 phage genomes and 34,981 prophages, which were before predicted from 18,872 prokaryotic genetic units (8,006 plasmids and 10,866 chromosomes). Using different comparative analysis based on shared orthologous proteins we calculated the functional similarity between all phage and prophage genomes. Phages and prophages that share a minimum set (jaccard 0.1) of orthologous genes were summarized in a graph. The full graph contains 196,354 edges (associations) between phage genomes, 3,514,604 edges between prophages and 90,659 edges connecting the phages and prophages. Statistical data and network analysis revealed that phages possessing large proteomes are less likely to be associated with a prophage. Further, using connectivity analysis we identified several pathogenic species like (*Salmonella enterica*, *Clostridium botulinum*, or *Bordetella pertussis*) that are highly connected in the network, meaning this species possesses a high number of prophages or prophages with high similarity to many other nodes in the network. The association network is able to uncover relations of phages, prophages and their host on different levels. Further it enables the ability, from the cluster of densely connected prophages, to predict putative phages genomes that are yet not sequenced but are of clinical interest for putative phage therapies.

---

### 037-FMV

Phylogenetic relationship of toxinogenic *C. difficile*-strains isolated from chicken meat samples processed in different cutting plants

\*J. Heise<sup>1</sup>, P. Witt<sup>1</sup>, C. Maneck<sup>1</sup>, S. Maurischat<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany

*Clostridioides difficile* (also *Clostridium difficile*) is the most common pathogen of nosocomial infections in humans which causes about 20-30% of antibiotic-associated diarrhea. A zoonotic transmission of *C. difficile* to humans is indicated by closely related isolates from humans and animals as well as the isolation of *C. difficile* from different food products. To estimate the exposure of humans with *C. difficile* via food, we screened different retail products of fresh chicken meat from Brandenburg/Berlin for the occurrence of *C. difficile*. We

revealed a relative high contamination rate of 14.1% in chicken meat with skin and detected PCR-ribotypes (RT) already found from CDI patients (e.g., RT 001, 002, 014) indicating that contaminated chicken meat may be a potential source of human infections. Moreover, we identified cutting plants with a higher risk of *C. difficile*-positive samples. To compare the relationship of the isolated strains from the concerning cutting plants over several months in 2018 and 2019, we analyzed them using NGS followed by core genome MLST. First results showed a contamination with the spore-forming pathogen over several months in same cutting plants. For instance, we detected a very similar strain (2-3 alleles distance) of the epidemiological RT 001 in chicken meat from a German cutting plant in January and March 2018. Next, strains of the RT 002 were detected in four cutting plants from Germany (Lower Saxony, Bavaria) and Austria, with an identical strain being persistent in one German cutting plant over 3 months. The ongoing contamination with same strains can be explained with a persistence of the pathogen within the cutting plant, or with a recurring entry of related strains e.g., originating from the same fattening farms. Further investigations of the concerning cutting plants and other levels of the food chain are necessary to clarify transmission routes and the contamination source.

---

### 038-FMV

Assessing the enteropathogenic potential of *Bacillus cereus* by means of secreted and surface-located markers

\*M. Kranzler<sup>1</sup>, N. Jeßberger<sup>2</sup>, C. Rademacher<sup>1</sup>, R. Dietrich<sup>2</sup>, T. Buchacher<sup>1</sup>, E. Märtlbauer<sup>2</sup>, M. Ehling-Schulz<sup>1</sup>

<sup>1</sup>Institute of Microbiology / University of Veterinary Medicine Vienna, Department of Pathobiology, Vienna, Austria

<sup>2</sup>Ludwig-Maximilians-Universität München, Lehrstuhl für Hygiene und Technologie der Milch, Oberschleißheim, Germany

**Question:** *Bacillus cereus* is one of the most relevant potential threats of food safety, due to its production of virulence factors and proteases. After ingestion of contaminated food, bacteria start in the intestine with the secretion of enterotoxins, which further lead to gastrointestinal symptoms. Apart from public health issues, food contaminations with *B. cereus* are also an economic problem, due to recalls of charges, and cannot totally avoided. Thus, a risk assessment of the toxic potential of *B. cereus* is crucial to make assumptions on the hazards of concerned food. However, the toxin gene profile as well as the amounts of secreted enterotoxins, such as the tripartite toxins Nhe (non-hemolytic enterotoxin), HBL (hemolysin BL) as well as the single component cytK (cytotoxin K), do not directly correlate with strain toxicity on Caco-2 cells. Also, commercially available tests on single toxin components can barely predict the actual toxic potential of *B. cereus* strains.

**Methods:** In order to determine potential markers correlating with pathogenicity, we combined phenomics with transcriptomics and proteomics (surfaceomics and secretomics).

**Results:** A holistic virulence assessment was generated considering stomach survival, adhesion, germination and motility as well as enterotoxin production. The predicted virulence correlated well with the secretion of NheB, sphingomyelinase and exoproteases, allowing us to establish a rapid and reliable diagnostic tool. Furthermore, so called "moonlighting proteins" – with primary functions in core intracellular pathways and secondary functions on the cell surface, mostly by binding to host proteins – were identified by surfaceomics as differentially expressed in high and low pathogenic strains, suggesting that these proteins might also contribute to the enteropathogenicity of *B. cereus*. First in

*vitro* studies revealed that *B. cereus* strains bind variably to different host proteins.

Conclusions: In this study, we identified toxicity-specific markers of *B. cereus* in the secretome and on the cell surface. Moonlighting proteins presumably play an additional, hitherto underestimated, role in pathogenesis.

### 039-FMV

Genetic and phenotypic characterization of *Vibrio cholerae* non-O1/non-O139 isolated from German retail seafood and coastal areas

\*S. Fleischmann<sup>1</sup>, E. Strauch<sup>2</sup>, I. Eichhorn<sup>3</sup>, L. Landoin<sup>4</sup>, R. Plickert<sup>5</sup>, T. B. Schille<sup>5</sup>, T. Alter<sup>1</sup>

<sup>1</sup>Freie Universität Berlin, Institute of Food Safety and Food Hygiene, Berlin, Germany

<sup>2</sup>Federal Institute for Risk Assessment (BfR), Department of Biological Safety, Berlin, Germany

<sup>3</sup>Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany

<sup>4</sup>Université de Brest, École Supérieure d'Ingénieurs en Agroalimentaire de Bretagne Atlantique, Brest, France

<sup>5</sup>Beuth Hochschule für Technik Berlin, University of Applied Sciences, Berlin, Germany

*Vibrio* (*V.*) *cholerae* belonging to the non-O1/non-O139 serogroups are present in German retail seafood as well as on the German coastal areas. These bacteria can be transmitted through the contact with contaminated seawater or the consumption of contaminated water and undercooked/raw seafood causing intestinal or extraintestinal infections. At present *V. cholerae* infections are rare in Germany but the risk of infections increases due to the steadily rising occurrence of *Vibrio* species caused by global warming.

In this study, 63 *V. cholerae* strains isolated from German retail seafood and 35 strains isolated from coastal areas of the German North Sea and Baltic Sea were analyzed using phenotypical and genotypical methods comparing these strains to 18 clinical non-O1/non-O139 strains as well as 5 pandemic O1/O139 strains to assess their pathogenic potential. Phenotypic assays were performed for hemolysin activity, human serum resistance and biofilm formation. The genetic characterization was carried out with regard to the major virulence-associated factors or markers of clinical *V. cholerae*, including the cholera toxin and the toxin-coregulated pilus as well as hemolysins, repeats-in-toxin toxins, pandemic islands VSP-1 /VSP-2 and the type III secretion system.

First results showed, that the main cholera virulence factors (*ctx*, *zot*, *ace*, *tcpA*, *rstR* and serogroups O1/O139) were absent in all non-O1/non-O139 *V. cholerae* strains. In contrast, genes encoding for the type III secretion system and the cholix toxin (*ChxA*) were present only in non-O1/non-O139 *V. cholerae* strains. Hemolysin genes like *hlyA* and *dth* conferring hemolysis on sheep blood agar, the resistance to human serum and the formation of biofilm were present in all groups. Additionally, a strong biofilm formation seems to be associated to non-clinical non-O1/non-O139 *V. cholerae* strains.

Against the background of global warming and an increasing seafood consumption in Germany (including the consumption of raw products like raw oysters and sushi), an additional testing for virulence genes beside *ctx* and serogrouping of O1/O139 should be considered in food diagnostics of *Vibrio* spp.

### 040-FMV

Long-term stability of an recombinant *Listeria monocytogenes*  $\Delta$ prfA strain in the VBNC state as an internal sample process control (ISPC)

\*C. Robben<sup>1</sup>, B. Bromberger<sup>2</sup>, D. Schoder<sup>2</sup>, \*P. Mester<sup>3</sup>, P. Rossmann<sup>4,2</sup>

<sup>1</sup>CD-Momiko, Unit of Food Microbiology, 1210, Austria

<sup>2</sup>Vetmeduni Vienna, Institute for milk hygiene, Vienna, Austria

<sup>3</sup>Christian Doppler Laboratory for Monitoring of Microbial Contaminants, Vienna, Austria

<sup>4</sup>Vetmeduni Vienna, CD-Momiko, Vienna, Austria

### Introduction:

Since its first implementation, PCR has been refined to a method that provides many opportunities for improved analysis of microorganisms. However, for its routine use in diagnosis, standardized validation protocols and guidelines, as well as supporting analytical elements (such as sample preparation), are necessary prerequisites. In order to assess and control the efficiency of each experimental step, a recombinant *Listeria monocytogenes*  $\Delta$ prfA (targeted deletion) strain containing a competitive artificial single-copy genomic target has been recently developed as an internal sample process controls (ISPCs). However, a drawback of ISPCs are the introduction of culturable bacterial cells which are closely related to the target of interest. One solution could be to use the ISPCs in the viable but non culturable (VBNC) state in which they are not able to proliferate, can be stored and are unlikely to resuscitate.

### Objectives:

In this study the long-term stability of *Listeria monocytogenes*  $\Delta$ prfA in the VBNC state was investigated for a period of 100 days.

### Material & Methods:

The VBNC state was investigated using a previously published induction protocol based on a salt/non-ionic detergent solution. A successful induction of the VBNC state was confirmed using cultivation methods, de-novo ATP generation, fluorescence microscopy and qPCR. Long-term stability of the VBNC ISPC cells was investigated for 100 days. VBNC cells were stored in three different buffer solutions and compared to living and killed cells.

### Results & Discussion:

The results of this study show that it is possible to store ISPC cells in the VBNC state for 100 days. The cells remained in the VBNC state throughout the investigated period and did not resuscitate. The qPCR signal from the VBNC ISPCs remained stable for 100 days, demonstrating their applicability to be used as controls.

### Conclusion:

Overall it could be shown that it is possible to store bacterial cells in the VBNC state for a period of 100 days and can be utilized as an ISPC throughout the storage period.

### 041-HYV

Data from a 5-year interventional study: a strict mask policy effectively reduces nosocomial influenza

\*A. Ambrosch<sup>1</sup>, D. Luber<sup>1</sup>, F. Klawonn<sup>2</sup>

<sup>1</sup>Krankenhaus Barmherzige Brüder, Institut für Labormedizin, Mikrobiologie und Krankenhaushygiene, Regensburg, Germany

Vaccination rates among health care workers (HCW) are low in Germany and may be a risk for transmission of influenza to patients in the clinical setting (1). Therefore, other preventive strategies should be taken into account to reduce the rate of nosocomial influenza. In this view, a study was conducted to compare two different hygiene regimens in which one focuses on a strict mask policy.

**Methods:** Data from hospitalized influenza patients (nosocomial rates / mortality) from the influenza season 2015 and 2016 (n=419) were compared to the seasons 2017 to 2019 (n=767): During the influenza season 2015 / 2016 patients were managed according to official recommendation ("standard protocol") (2). During the season 2017 to 2019, a strict mask policy for HCW was introduced ("mask policy") containing a mandatory wearing of surgical masks during the whole shift when 3 or more Influenza inpatients were at ward.

**Results:** During the season with a strict "mask policy" compared to the "standard protocol", the rate of nosocomial influenza infections decreased from 21.7 % to 9.97 % ( $p < 0.001$ , OR 0.51) being most effective for nosocomial influenza A transmission (Influenza A: 24.3 % to 12.8 %,  $p = 0.014$ ; influenza B: 8.6 % to 5.5 %,  $p = 0.118$ ). Consequently, mortality from nosocomial influenza was also significantly reduced ( $p < 0.001$ ). The vaccination rate of HCW only slightly increased during the observational period from 17 % (2015 / 2016) to 29 % (2017 to 2019) for nurses and 42 % and 53 % for doctors, respectively.

**Conclusion:** a strict mask policy for HCW is highly effective and reduces nosocomial influenza infection by nearly 50 % in the clinical setting. Thus the present data clearly focuses on the eminent role of hospital staffs in the transmission of nosocomial influenza.

#### References:

- Neufeind et al, Epi Bull 2018, 32, 313-321
- [https://www.rki.de/DE/Content/Infekt/EpidBull/MerkbLaetter/Ratgeber\\_Influenza\\_saisonal.html](https://www.rki.de/DE/Content/Infekt/EpidBull/MerkbLaetter/Ratgeber_Influenza_saisonal.html)

---

#### 042-HYV

##### Nosocomial influenza – what really matters?

\*N. Metzger<sup>1</sup>, M. Kaase<sup>1</sup>, A. E. Zautner<sup>2</sup>, D. Fenz<sup>1</sup>, S. Scheithauer<sup>1</sup>

<sup>1</sup>Universitätsmedizin Göttingen, Institut für Krankenhaushygiene und Infektiologie, Göttingen, Germany

<sup>2</sup>Universitätsmedizin Göttingen, Institut für medizinische Mikrobiologie, Göttingen, Germany

#### Introduction

Though nosocomial influenza (NI) poses a challenge to affected patients as well as to hospitals, the main transmission route in this setting is yet unclear. Possible sources are infected patients, healthcare workers (HCW) and visitors.

#### Objectives

Defining the proportion of NI patients (NIPs) with a traceable transmission (TM) through another patient.

#### Material & methods

This single-center retrospective study included all patients with laboratory-confirmed influenza (LCI) during the seasons 2016/17, 2017/18 and 2018/19.

#### Definitions:

- NIP: first positive sample taken >2d after admission without any signs or symptoms of influenza on admission.
- Contagious patient (CP): 1d before first pos. sample was retrieved till 7d thereafter.
- Donor: CP infected with the same influenza species (A/B), in the same room or ward with the NIP 2 to 5 d prior the NIPs first positive sample date.

A TM was assumed, if there was at least one plausible donor. Underdiagnosing was corrected by identifying patients classified by ICD-10 J09.- to J18.- who shared the ward with NI for the relevant time. LCI patients were isolated in single rooms or cohorted. Patients in the ICU were considered being immobile.

#### Results:

- A total of 426 patients with LCI were identified, thereof 55(13%) patients with NI.
- 27/55 (49%) NIP had a plausible TM hypothesis through another patient.
- Of these 27, 20 NIPs (74%) shared only the ward with their suggested donor(s). Furthermore, the only plausible donor of 11 of those 27 (41%), was isolated or immobile.
- the 41 possible donors were treated as follows: 29(71%) neuraminidase inhibitor, 37(90%) antibiotics, 26(63%) both
- 14/28 NIP (50%) without feasible donor shared their room with a patient with virus-caused (N=2) or unspecified (N=11) pneumonia classified by ICD-10.

#### Conclusion:

About 74% of the plausible NI transmission events took place in the same ward, not room, and in about 40% of these TMs, the only donor was isolated or immobile. Thus, the most likely way of transmission seems to be contact transmission via HCW or droplet transmission by unidentified patients or HCW or visitors. However, underdiagnosing seems to be of importance

---

#### 043-HYV

Reduction of surgical site infections after the Implementation of a new bundle of infection prevention measures by the infection control physician

\*M. Neuwirth<sup>1,2</sup>, R. Otchwemah<sup>1,3,2</sup>, F. Mattner<sup>1,2</sup>

<sup>1</sup>Kliniken der Stadt Köln gGmbH, Institut für Hygiene, Köln, Germany

<sup>2</sup>Universität Witte- Herdecke, Lehrstuhl für Hygiene und Umweltmedizin, Köln, Germany

<sup>3</sup>Kliniken der Stadt Köln gGmbH, Klinik für Orthopädie, Unfallchirurgie und Sporttraumatologie, Köln, Germany

## Introduction and Objectives

In the BMG-funded project "HygArzt" (ZMVI1-2516FSB111), the effects of a new bundle of infection prevention measures on the infection rates (IR) of nosocomial infections (NI) and in particular on surgical site infections (SSI) implemented by the infection control physician was investigated on three orthopedic/trauma surgery general wards.

## Methods

As study design, a pre-/post- intervention study was chosen. Both, trauma surgery and orthopedic patients were included. As intervention, a bundle was implemented which included: Decolonization of patients before surgery, examination of preoperative antibiotic prophylaxis, standardized wound and fixator care, treatment of closed surgical wounds with remanent skin disinfectants in the operating theatre and closed incision prophylactic negative pressure wound therapy for high-risk wounds. To identify NI, clinical signs of infection were recorded according to KISS and CDC definitions. Additionally, an active surveillance, which included morning rounds three times a week in order to detect undocumented signs of infection was performed.

## Results

During the pre-phase (1141 surgeries) 61 NIs were recorded (IR 5.3% (CI 95% 4.0; 6.6)) and 44 SSIs (3.1% (CI 95% 1.5; 3.2)). In the post-phase (1546 surgeries) 35 NIs were recorded (IR 2.3% (CI 95% 1.1; 2.7)) and 26 SSIs (1.7% (CI 95% 1.1; 2.3)). The post-phase showed significantly lower relative risks for NIs ( $RR=0.43$  (CI 95% 0.28; 0.64),  $p<0.001$ ) and SSIs ( $RR=0.44$  (CI 95% 0.27; 0.70),  $p<0.001$ ) compared to the pre-phase. A subgroup analysis for SSI of trauma patients (pre: (745 surgeries, 29 SSIs, IR 3.9% (CI 95% 2.5; 5.3)); post: (937 surgeries, 19 SSIs, IR 2.0% (CI 95% 1.1; 2.9))) and orthopedic patients (pre: (696 surgeries, 15 SSIs, IR 2.2% (CI 95% 1.1; 3.3)); post: (608 surgeries, 8 SSIs, IR 1.3% (CI 95% 0.4; 2.2))) revealed a significant effect only in trauma patients ( $RR=0.52$  (CI 95% 0.29; 0.92),  $p=0.02$ ).

## Conclusion

NI rates and SSI rates could be reduced by a new infection control bundle in orthopedic and trauma patients implemented by an infection control physician. The effect was pronounced and significant in trauma patients.

## 044-HYV

Surveillance of surgical site infections – methodical comparison of IQTIG- and KISS- strategy

\*S. Rittmeier<sup>1</sup>, R. M. Waeschle<sup>2</sup>, T. Artelt<sup>1</sup>, P. Fehling<sup>1</sup>, I. Thalmann<sup>1</sup>, A. Suckow<sup>3</sup>, S. Scheithauer<sup>1</sup>

<sup>1</sup>University Medical Center Goettingen, Institute for Infection Control and Infectious Diseases, Goettingen, Germany

<sup>2</sup>University of Goettingen, Department of Anaesthesiology, Emergency and Intensive Care Medicine, Goettingen, Germany

<sup>3</sup>University Medical Center Goettingen, Quality and Risk Management Department, Goettingen, Germany

## Introduction

In 2017 the IQTIG surveillance of surgical site infections (SSI) for benchmark and quality improvement purposes was

introduced. So far, no comparison of the IQTIG vs. the KISS surveillance is available.

## Objectives

Our study compares IQTIG and KISS surveillance results in order to define the comparability and to identify factors associated with disagreement.

## Materials and Methods

All patient in 2018 qualifying for coding according to IQTIG algorithms were enrolled. Coding for IQTIG was performed by physicians, coding for KISS was performed by one investigator previously trained in KISS surveillance and blinded to the IQTIG results. In cases of uncertainty two infection control experts judged the coding.

## Results

In 2018 in 1,238 cases (not all underwent surgery) an electronic evaluation form according to IQTIG was triggered, thereof 1,118 (88%) were assessed by both strategies. For the remaining 120 patient cases no IQTIG evaluation was performed.

In 250 of the doubled assessed cases (22%) a SSI was diagnosed in each evaluation strategy. In 933 (83%) IQTIG and KISS strategy revealed the same results ("S"). Thus, 185 cases (17%) were evaluated with different results ("D"). Within these 185 cases, 91 (49%) were evaluated positively for SSI by IQTIG and negatively by KISS, whereas 94 cases (51%) were evaluated negatively for SSI by IQTIG and positively by KISS, respectively.

Risk factor analysis for different coding results ("D" compared with "S") revealed, that

1) in the group of differently assessed patients the number of infections other than SSI seems to be higher (26/185; 14% vs. 88/933; 9%;  $p=0.062$ )

2) differently assessed patients with surgical procedure were coded significantly less often by the department performing the surgical process (131/163; 80% vs. 551/634; 87%;  $p=0.045$ )

3) differently assessed patients with surgical procedure significantly more often had an ASA Score III or IV (93/163; 57% vs. 292/634; 46%;  $p=0.014$ )

## Conclusion

The IQTIG surveillance strategy and the KISS strategy showed a high rate of consensus. Some of the results identified shortcomings which may be corrected and thus potentially improving IQTIG results.

## 045-HYV

Implementation of universal preoperative decolonization with polihexanide in primary joint arthroplasty and its effect on surgical site infections. A multicentre prospective cohort study.

\*T. Kramer<sup>1</sup>, B. Wandhoff<sup>2,3</sup>, C. Schröder<sup>2</sup>, U. Nöth<sup>3</sup>, R. Krause<sup>4</sup>, B. Schmidt<sup>4</sup>, S. David<sup>5</sup>, E. E. Scheller<sup>6</sup>, M. Behne<sup>2,1</sup>, P. Gastmeier<sup>2</sup>

<sup>1</sup>Charité - University Medicine Berlin, Institute for hygiene and environmental medicine, Berlin, Germany



<sup>2</sup>Charité Universitätsmedizin Berlin, Hygiene und Umweltmedizin, Berlin, Germany

<sup>3</sup>Evangelisches Waldkrankenhaus Spandau, Orthopedic surgery and traumatology, Berlin, Germany

<sup>4</sup>Oberlin Klinik, Potsdam, Germany

<sup>5</sup>Evangelisches Krankenhaus Paul Gerhardt Stift, Lutherstadt Wittenberg, Germany

<sup>6</sup>Evangelisches Krankenhaus Hubertus, Berlin, Germany

Surgical site infections (SSI) are one of the most severe complications in joint arthroplasty. Decolonization measures prior to elective orthopedic surgeries have shown to reduce the risk of SSI especially in patients colonized *Staphylococcus aureus*.

Does implementation of universal decolonisation with polihexanide containing substances in patient with primary joint arthroplasties have an effect on SSI-rates.

## Methods

Between January 2015 and December 2018 patients scheduled for hip or knee joint arthroplasty in 5 participating orthopedic centres were included. From January 2017 patients received polihexanide containing decolonization sets. Patients were instructed to perform a 5 day decolonization regimen starting 4 days prior to surgery. SSI were recorded according to CDC criteria.

## Results

During the study period, 14189 patients received native joint replacement. After Intervention was implemented 7633 surgeries were performed, while 4437 decolonization sets were distributed to patients. 1869 patients consented to participate in the study and provide detailed feedback on compatibility and compliance. Overall SSI rate was 0.85 per 100 surgeries prior to introduction of the decolonization, while it was 0.96 per 100 surgeries during the period of decolonization and 0.59 per 100 surgeries in those using the decolonization set. SSI rates due to *Staphylococcus aureus* (SAU) were 0.24 per 100 surgeries, 0.21 per 100 surgeries and 0.05 per 100 surgeries respectively. Surgery after implementation of the intervention was identified as an independent protective factor against SAU-SSI (OR .57 ; 95% CI: .32-.99) in our multivariable regression analysis

## Conclusions

A universal decolonization strategy with polyhexanide reduces *S. aureus* SSI rates in patients with elective joint arthroplasty. Further evaluations are needed.

---

## 046-HYV

Use of clinical microbiological methods to differentiate nosocomial and community acquired pneumonias during a nosocomial outbreak within the same hospital

\*C. Lück<sup>1</sup>, B. Langer<sup>2</sup>, K. Lück<sup>1</sup>, S. Pleischl<sup>3</sup>, M. Exner<sup>3</sup>, B. Hornei<sup>4</sup>

<sup>1</sup>Med.Fakultät der TU Dresden, Institut für Medizinische Mikrobiologie, Dresden, Germany

<sup>2</sup>Hygiene-Institut des Ruhrgebietes, Gelsenkirchen, Germany

<sup>3</sup>Universität Bonn, Institut f. Hyg. u. Öfftl. Gesundh., Bonn, Germany

<sup>4</sup>Institut für Laboratoriumsmedizin und Klinische Mikrobiologie, Oberhausen, Germany

Legionella is found in natural aquatic and soil habitats. More than 90% of LD cases are caused by Legionella pneumophila serogroup (sg 1). The detection of the source of infection needs the detection of the causative strain(s) in respiratory samples.

During a nosocomial outbreak in 2017, 18 patients with pneumonia confirmed by Legionella urinary antigen detection as caused by Legionella pneumophila serogroup (sg1) were treated in a German hospital. Based on the incubation period six cases were defined as definitely nosocomial (NAP)>10 days stay in the hospital) and seven cases as possible nosocomial (3 to 9 days stay in the hospital). The remaining six patients were defined as community acquired (CAP) since they were admitted with the pneumonia or had a short stay within the hospital. Half of the patients were male, half were female aged from 40 to 89 years. Two nosocomial and two community acquired cases died.

*L. pneumophila*, sg 1, monoclonal antibody type (MAbtype) Knoxville, sequence type (ST) 9 was confirmed as the epidemic strain.

For the detection of *L. pneumophila* soluble antigen in urine, specimens were tested using a rapid immunochromatographic card. In positive urine samples a direct monoclonal typing approach was applied.

Respiratory samples were processed for PCR and culture. To define the sequence type directly from clinical samples nested SBT was performed on PCR positivesamples. All respiratory samples were cultured. Isolated Legionella strains were typed by monoclonal antibody typing and sequence based typing .

From 10 patients (four with CAP and six with NAP) respiratory samples were investigated by culture and PCR. In the six nosocomial cases, the epidemic strain was detected by culture or direct sequence based typing (dSBT) in respiratory samples. In further three nosocomial cases, the causative strain of MAbtype Knoxville was confirmed by MAb based urinary antigen testing. On the opposite in four community-acquired cases, other strains were detected.

This success was due largely to the good working relationships between staff in the organizations involved which led to the prompt referral of respiratory samples.

---

## 047-EPAV

**Assigning a putative role in energy-conservation to the membrane subunits of the formate hydrogenlyase complex from the enterobacterium *Trabulsiella guamensis*.**

S. Zantop Linares<sup>1</sup>, U. Lindenstrauß<sup>1</sup>, \*C. Pinske<sup>1</sup>

<sup>1</sup>Martin-Luther University Halle-Wittenberg, General Microbiology, Halle, Germany

The formate hydrogenlyase (FHL) complex is phylogenetically related to the proton-translocating complex I of the respiratory chain, but catalyzes the disproportionation of formate to H<sub>2</sub> and CO<sub>2</sub> during mixed-acid fermentation. The soluble domain is located in the cytoplasm and comprises the activities of a [NiFe]-hydrogenase and a formate dehydrogenase and is further attached to a membrane arm. Two versions of FHL exist that differ in the number of membrane subunits, which suggests differences in their involvement in energy-conservation.

We investigated the five membrane-subunit version of FHL<sub>Tg</sub> from *Trabulsiella guamensis* through heterologous expression in *Escherichia coli* and used membrane-truncated versions of the complex to determine the order of the membrane subunits and their susceptibility to the protonophore CCCP and the sodium ionophore EIPA during H<sub>2</sub>-evolution on a Clark-type hydrogen electrode.

The simultaneous loss of H<sub>2</sub>-dependent reduction of the redox dye benzyl viologen but retention of the reverse reaction (H<sub>2</sub>-production from the redox dye methyl viologen) in crude extracts, indicated that the reaction direction is regulated. Loss of the membrane domain during enrichment of the FHL<sub>7g</sub> complex correlated with loss of this directional regulation. Results with generation of membrane-domain variants indicated that the complex has an inverted membrane domain organization compared to complex I. While addition of CCCP or catalysis in high-salt buffer reduced the FHL<sub>7g</sub> reaction slightly, a combination of both or CCCP with EIPA completely inhibited catalysis. Interestingly, and in contrast to the homologous system, in the *E. coli* system the FHL<sub>7g</sub> complex showed increased activity with high salt and CCCP, but only when glucose was used as substrate. Similarly, deletion of two of the distal subunits HyfF or HyfD caused an increased rate of H<sub>2</sub> production after CCCP treatment, while loss of HyfE caused a rate-reduction in the presence of CCCP.

Taken together these data shed new light on the role of the membrane arm of FHL<sub>7g</sub> in energy conservation and catalysis by the complex. A model for proton and sodium flux through the membrane domain can be deduced.

#### 048-EPAV

Pyrite formation from FeS and H<sub>2</sub>S: a novel microbial energy metabolism

J. Thiel<sup>1</sup>, J. Byrne<sup>2</sup>, A. Kappler<sup>2</sup>, B. Schink<sup>1</sup>, \*M. Pester<sup>3</sup>

<sup>1</sup>University of Konstanz, Dept. of Biology, Konstanz, Germany

<sup>2</sup>Eberhard Karls University of Tübingen, Tübingen, Germany

<sup>3</sup>Leibniz Institute DSMZ, Dept. of Microorganisms, Braunschweig, Germany

The exergonic reaction of FeS with H<sub>2</sub>S to form FeS<sub>2</sub> (pyrite) and H<sub>2</sub> was postulated as an early form of energy metabolism on primordial Earth. With an annual production of more than 5 million tons, pyrite is also today the stable end product of iron compounds reacting with sulfide in reduced sediments. However, the mechanism of sedimentary pyrite formation is still being debated. We present enrichment cultures, which grew with FeS, H<sub>2</sub>S, and CO<sub>2</sub> as their sole substrates to produce pyrite and methane [1]. Pyrite formation followed a clear biological temperature profile with maximum activity at 28°C. Methane formed concomitantly with pyrite and exhibited the same temperature dependence. Addition of either penicillin or 2-bromoethanesulfonate inhibited both pyrite and methane production, indicating a syntrophic coupling of pyrite formation to methanogenesis. This hypothesis was supported by a 16S rRNA gene-based phylogenetic analysis, which identified at least one archaeal and five bacterial species. The archaeon was closely related to the hydrogenotrophic methanogen *Methanospirillum stamsii* while the bacteria were most closely related to sulfate-reducing Deltaproteobacteria, as well as uncultured Firmicutes and Actinobacteria. We identified a novel type of microbial metabolism driven by FeS transformation to pyrite that could sustain part of the deep biosphere in sediments and which may serve as a model for a postulated primordial iron-sulfur world.

[1] Thiel, J., Byrne, J.M., Kappler, A., Schink, B., and Pester, M. (2019) Pyrite formation from FeS and H<sub>2</sub>S is mediated through microbial redox activity. *Proc Natl Acad Sci USA*. **116**: 6897-6902.

#### 049-EPAV

Citrate cleavage through citrate synthase: the reversed oxidative TCA cycle and its distribution

\*L. M. Steffens<sup>1</sup>, E. Pettinato<sup>1</sup>, A. Mall<sup>1</sup>, I. Berg<sup>1</sup>

<sup>1</sup>Westfälische Wilhelms-Universität Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany

*Desulfurella acetivorans* is a sulfur-reducing deltaproteobacterium capable to grow autotrophically with H<sub>2</sub> or heterotrophically by acetate oxidation using the reversed oxidative tricarboxylic acid (roTCA) cycle [1]. Citrate cleavage (reductive direction) and citrate synthesis (oxidative direction) are both catalyzed by ATP-independent citrate synthase (CS), whereas the classical ATP-dependent citrate lyase is absent [1, 2]. *D. acetivorans* harbours three CS, two of them we characterized biochemically. While one isoenzyme is a typical citrate synthase, whose *K<sub>M</sub>* value to citrate (1 mM) is nevertheless close to the intracellular citrate concentration in autotrophically grown cells (1.4 mM) [1], the second CS homolog was a promiscuous enzyme with citrate and methylcitrate synthase activities [3]. The comparison of CS activity in *D. acetivorans* cell extracts and the activity of heterologously produced CS suggests that this enzyme constitutes ~20% of cellular protein. Indeed, the only specific recognizable feature of the roTCA cycle in bacteria is its dependence on unusually high activities of CS and malate dehydrogenase (MDH). It allows to overcome unfavourable kinetic and thermodynamic properties of citrate cleavage reaction catalyzed by CS. As codon usage bias strongly correlates with the protein/transcript level, we examined whether the presence of the roTCA cycle can be predicted by analysis of the codon usage of the corresponding genes. Although the results of the analysis were not ambiguous for some studied microbial groups, we identified several bacterial species that potentially use the roTCA cycle. In the case of *Hippaea maritima* (Desulfurellaceae), we were able to confirm very high activities of CS and MDH, demonstrating that this type of bioinformatic analysis opens a possibility to recognize otherwise invisible metabolic pathways. Furthermore, our results suggest that the emergence of the roTCA cycle does not require specific adaptation of CS to catalyze the thermodynamically unfavourable citrate cleavage reaction.

[1] A. Mall *et al.*, *Science* **359**, 563-567 (2018).

[2] T. Nunoura *et al.*, *Science* **359**, 559-563 (2018).

[3] E. Pettinato *et al.*, poster (2020).

#### 050-EPAV

**Spotlight on the energy harvest of electroactive microorganisms: The impact of the redox potential of the solid terminal electron acceptor**

\*B. Korth<sup>1</sup>, F. Harnisch<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany

#### Introduction

Electroactive microorganisms (EAM) are capable of performing extracellular electron transfer (EET) representing a unique metabolic trait. The terminal electron acceptor (TEA) is solid and can be, e.g., metal ore or anode. Although EAM attracted considerable interest, their thermodynamics is still unexplored. However, assessing the energy fluxes during EET is a prerequisite for deciphering their ecological role and for leveraging their technological potential.

#### Objectives

Due to the peculiarity of their electron-transport chain (ETC), the energy harvest of EAM is restricted. Metabolically received electrons are transferred across the cell membrane, while a share of ETC components is located in the periplasm and at the outer membrane. Thus, these cannot contribute to the generation of proton gradient across the inner membrane subsequently utilized for ATP synthesis. Consequently, the question arises, to which extent the potential of the TEA influences the energy harvest of EAM.

## Materials & methods

A previously developed model for electroactive biofilms<sup>1</sup> combining thermodynamic calculations on microbial growth, electrochemical, chemical, and physical equations was applied for deciphering the impact of the anode potential on the energy harvest of the model EAM *Geobacter sulfurreducens*.

## Results

Energy harvest of *G. sulfurreducens* saturates at anode potential of 0.2 V (vs. SHE) representing full exploitation of the thermodynamic frame. The anode potential affects EET rate and in turn the energy-yielding reaction (i.e., substrate oxidation) by changing reaction conditions. However, this is solely a kinetic effect, the thermodynamic frame of this EAM is not influenced by the potential of the solid TEA.

## Conclusion

Assessing the energy harvest of EAM requires an adapted approach compared to classical thermodynamic calculations. Instead of calculating the energy difference between electron donor and terminal electron acceptor, the energy difference between electron donor and an intracellular electron carrier contributing to the proton gradient should be calculated<sup>2</sup>.

<sup>1</sup>Korth et al., 2015, *Bioelectrochemistry*

<sup>2</sup>Korth et al., 2019, *Front Microbiol*

## 051-EPAV

The author has not agreed to a publication.

## 052-EPAV

### Uncovering key players in anaerobic benzene degradation under nitrate-reducing conditions from over 2000 metagenome-assembled genomes in a microcosm experiment

\*F. Borim Corrêa<sup>1,2</sup>, S. Eziuzor<sup>3</sup>, S. Peng<sup>3,4</sup>, J. Schultz<sup>5</sup>, L. Adrian<sup>3</sup>, S. Kleinstüber<sup>1</sup>, C. Vogt<sup>3</sup>, U. Nunes da Rocha<sup>1</sup>

<sup>1</sup>Helmholtz-Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>University of Leipzig, Department of Computer Science and Interdisciplinary Center of Bioinformatics, Leipzig, Germany

<sup>3</sup>Helmholtz-Centre for Environmental Research - UFZ, Department of Isotope Biogeochemistry, Leipzig, Germany

<sup>4</sup>Chongqing University, Department of Environmental Science, Chongqing, China

<sup>5</sup>Universidade Federal do Rio de Janeiro, Departamento de Microbiologia Geral, Rio de Janeiro, Brazil

A community mineralizing benzene under nitrate-reducing conditions, originally enriched in sand-filled bioreactors percolated with groundwater at a benzene-contaminated aquifer, has been maintained in laboratory microcosms for many years. A controlled succession experiment using multi-

omics approaches could reveal microbial interactions and bioindicators for benzene degradation. In this study, we monitored the succession of species in this community during the degradation of benzene under nitrate-reducing conditions. The laboratory microcosms inoculated with our benzene-degrading community were fed with benzene (25% <sup>13</sup>C). We sampled the solid (sand) and liquid phases from the microcosms at five time points: experiment set up (T0), beginning of mineralization (T1), early log-phase (T2), late log-phase (T3) and end of experiment (T4). After DNA extraction, we sequenced metagenomes for all samples (150 bp paired-end Illumina reads, minimum 20 million reads per library). We recovered 2006 good quality (low to no contamination) metagenome-assembled genomes (MAGs), spanning 144 bacterial and 3 archaeal unique taxa. The most recovered prokaryotic MAGs belong to the orders Betaproteobacteriales (320), Anaerolineales (192), Rhizobiales (128) and Ignavibacteriales (86). From Archaea, we recovered MAGs from the phyla Microarchaeota (3), Nanoarchaeota (2) and Thermoplasmatota (2). Over 5.5 million unique proteins were predicted. Many enzymes related to metabolic pathways involved in benzene mineralization were identified. Those include also enzymes related to metabolism of inorganic nitrogen. For example, enzymes related to the anaerobic oxidation of ammonium, indicating a likely active intake of nitrite by ANAMMOX bacteria. Currently, we are conducting stable isotope probing of the proteome (SIP-proteomics) from all samples and performing functional annotation of the recovered MAGs. Tracking labeled proteins will indicate not only the key players in the metabolism of benzene but also how carbon flows through the microbial community. In the end, we expect to better understand the mechanisms underlying anaerobic benzene mineralization under nitrate-reducing conditions.

## 053-EPAV

Turnover of intermediates and the active microbiota during degradation of <sup>13</sup>C-maize in a biogas digester by RNA-based stable isotope probing (RNA-SIP)

\*N. Ruppel<sup>1</sup>, K. Schäfer<sup>1</sup>, F. Buegger<sup>2</sup>, M. A. Horn<sup>1</sup>

<sup>1</sup>Institute of Microbiology / Leibniz University Hannover, Hannover, Germany

<sup>2</sup>Helmholtz Zentrum, Muenchen, Germany

Biogas is an important renewable energy resource and is produced during the anaerobic food chain. Complex biopolymers are degraded by a consortium of microorganisms. Numerous microbial taxa interact and perform hydrolysis, many primary and secondary syntrophic fermentations producing acetate and H<sub>2</sub>/CO<sub>2</sub>. Syntrophs are crucial for converting intermediates to methane precursors. Methanogens are the terminal electron sink and convert acetate and H<sub>2</sub>/CO<sub>2</sub> into CH<sub>4</sub>. However, substrate specific key players remain to be identified as well as rates of interconnected pathways, which are crucial for process stability and CH<sub>4</sub> yield. Thus, a time-resolved RNA-SIP experiment was performed using material from a one-stage, thermophilic, maize-fed biogas digester. <sup>13</sup>C-maize (97 atom.-% <sup>13</sup>C) was supplemented to anoxic digestate incubations at 50 °C as one-time dose, and labeled intermediates and products quantified regularly by LC-MS and GC-IRMS. Six time points were chosen for density gradient centrifugation of RNA to identify active assimilators of maize derived <sup>13</sup>C by Illumina MiSeq. Fully <sup>13</sup>C-labeled important process intermediates were acetate and propionate, which increased immediately after <sup>13</sup>C-maize addition, with maximal concentrations after 5 h and 11 h, respectively. Accumulation of other labeled organic acids was not observed. <sup>13</sup>CH<sub>4</sub> and <sup>13</sup>CO<sub>2</sub> increased linearly without appreciable delay. Production of <sup>13</sup>CO<sub>2</sub> decreased after 17 h. The highest H<sub>2</sub> concentration of 150 ppm was

measured after 15 h and was subsequently reduced. Organisms within the phylum Firmicutes were always <sup>13</sup>C-labeled. Clostridiaceae and Dysgonamonadaceae were <sup>13</sup>C-labeled after 3 h, suggesting association with primary fermentations, and Halanaerobiaceae as well as an uncultured family within the Clostridia were <sup>13</sup>C-labeled after 9 h. Putative secondary fermenting syntrophs of the genera *Syntrophomonas*, *Gelria* and *Tepidanaerobacter* were labeled after 39 h. In conclusion, we identified hitherto known and unknown thermophilic key players associated with carbon turnover in such types of bioreactors, including key syntrophs that are crucial to functioning of the anaerobic food chain.

---

#### 054-EPAV

A new metabolic pathway for the conversion of pentoses in gut microbiota

\*L. Garschagen<sup>1</sup>, U. Deppenmeier<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Institute Microbiology and Biotechnology, Bonn, Germany

The gut microbiota plays a decisive role for the human immune system and supports digestion by expanding the substrate spectrum of the host. The majority of the human intestinal bacteria belong to the Phyla Firmicutes or Bacteroidetes. These organisms are responsible for the breakdown of fibers, which are not degraded by human digestive enzymes. Although the analysis of central metabolic pathways of gut microbes is increasingly gaining importance, it has never been investigated how these species metabolize pentoses, the monomeric units of e.g. the important fiber hemicellulose. Bioinformatic analyses revealed that a transaldolase as the key enzyme of the regular pentose phosphate pathway (PPP) is absent in many species of the human gut (e.g. *Bacteroides*, *Prevotella*, *Faecalibacterium* and *Ruminococcus* species). To find evidence for an alternative pentose conversion pathway, we analyzed one of the main players in the colon, *Prevotella copri*, for its potential to degrade xylose. We could show that a pyrophosphate-dependent phosphofructokinase (PPI-PFK) in combination with a fructose-1,6-bisphosphate-aldolase (FBP-aldolase) was able to replace the transaldolase reaction. In summary, this newly discovered pathway for the degradation of C5-sugars differs significantly from the well-known pentose metabolism of common bacteria and represents a mixture of the PPP and a reversed Calvin cycle. Bioinformatic data showed, that in the absence of the transaldolase, the gene encoding the PPI-PFK can function as a marker that indicates the presence of this alternative pathway for pentose degradation in gut bacteria.

---

#### 055-MBTV

Lactic acid bacteria - The sCapedGoat for effective medium-chain carboxylate production

\*J. Lambrecht<sup>1</sup>, N. Cichocki<sup>1</sup>, F. Schattenberg<sup>1</sup>, S. Kleinsteinuber<sup>1</sup>, H. Harms<sup>1</sup>, S. Müller<sup>1</sup>, H. Sträuber<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

Question: The carboxylate platform is a promising technology for substituting petrochemicals in the provision of specific platform chemicals and liquid fuels. It includes the microbial chain elongation of short-chain fatty acids to form the more valuable medium-chain variants. We investigated: How does the pH value in the bioreactor shape the microbial community? How does the microbiome in turn influence the production process? And does the microbiome show dynamic behaviour during the anaerobic fermentation that influences the product formation?

Methods: We operated a 15 L open system continuous stirred tank reactor for 241 days and combined flow cytometric short interval monitoring, cell sorting and 16S rRNA gene amplicon sequencing to investigate microbiome structure and dynamics.

Results: Flow cytometry recorded 31 sub-communities with cell abundances varying over 89 time points. It revealed a highly dynamic community, whereas the sequencing analysis displayed a mostly unchanged core community. Eight key sub-communities were linked to caproate or caprylate production (rS > |±0.7|). Amongst other insights, sorting and subsequently sequencing these sub-communities revealed the central role of *Bifidobacterium* and *Olsenella*, two genera of lactic acid bacteria that drove chain elongation by providing additional lactate, serving as electron donor. Caproate and caprylate titres of up to 6.12 g/L and 1.83 g/L, respectively, were achieved. Caproate production was optimal at pH 5.5 and connected to lactate-based chain elongation, while caprylate production was optimal at pH 6.25 and linked to ethanol utilisation.

Conclusions: High-titre medium-chain fatty acid production with complex substrate is possible without the addition of external electron donors. This result will greatly ease scaling and profitable implementation of the process. The pH value influenced the substrate utilisation and product spectrum by shaping the microbial community. Flow cytometric single cell analysis enabled the fast, short interval analysis of the microbial community and was coupled with 16S rRNA gene amplicon sequencing to reveal the pivotal role played by lactate-producing bacteria.

---

#### 056-MBTV

Genetic analysis of cellulose synthesis in acetic acid bacteria

\*M. Bimmer<sup>1</sup>, G. Wanner<sup>2</sup>, W. Liebl<sup>1</sup>, A. Ehrenreich<sup>1</sup>

<sup>1</sup>Technical University Munich, Chair of Microbiology, Freising, Germany

<sup>2</sup>Ludwig Maximilians University, Plant Sciences, Planegg - Martinsried, Germany

Among strains of the acetic acid bacteria genus *Komagataeibacter* are high microcrystalline cellulose producers. Due to its high capacity to store water and its mechanical strength, bacterial cellulose (BC) is a very versatile biomaterial. The strains contain three cellulose synthase operons of different complexities. All operons consist of at least the synthase itself and a channel protein. Several diguanylate cyclases (DGC) catalyze the formation of c-di-GMP that induces biofilm formation after binding to the c-terminal PilZ domain of AcsAB.

This study aims on the analysis of the role of respective cellulose synthase operons and their regulation. Several markerless multi-deletion mutants of elements of the cellulose synthase operons and the DGCs as well as fluorescent reporter fusions were constructed and used to quantify produced cellulose as well as to assess the role of the operons for biofilm formation by analyzing structural differences in biofilm composition as well as by fluorescence microscopy and scanning electron microscopy. The genetic system we developed for *Komagataeibacter* strains is, to our best knowledge, the only system applicable to construct markerless mutants in cellulose producing prokaryotes.

*K. xylinus* ATCC 53582 strain shows high quantities of BC synthesis, deletion of *dgc1* reduced the amount of produced cellulose significantly, while deletion of *dgc2* abolishes cellulose formation completely. In *K. hansenii* ATCC 23769 a

significantly lower amount of cellulose was measured after deletion of elements of the AcsAB operon. The importance of AcsAB for biofilm formation, compared to AcsAB2 and AcsAB3 was supported by SEM data.

---

## 057-MBTV

### Construction of an *Aspergillus oryzae* triple amylase deletion mutant as a chassis to produce industrially relevant amylases using multiplex CRISPR/Cas9 editing technology

\*G. Haeger<sup>1</sup>, A. Grankin<sup>2</sup>, M. Wagner<sup>2</sup>

<sup>1</sup>FH Aachen, Institute of Nano and Biotechnologies (INB), Jülich, Germany

<sup>2</sup>Hochschule Niederrhein, Biotechnology, Krefeld, Germany

*Aspergillus oryzae* is an industrially relevant organism for the secretory production of heterologous proteins. Due to high level secretion of the native  $\alpha$ -amylase, *A. oryzae* is especially interesting for the expression of heterologous amylases. The activities of heterologously produced amylases, however, cannot be quantified directly within the culture broth due to the high background activity of the native  $\alpha$ -amylase. This background activity is caused by the gene products of *amyA*, *amyB* and *amyC*. Deletion of all three amylase encoding genes led to the complete loss of amylase activity in the culture supernatant [1]. For heterologous expression target genes are usually randomly integrated into *A. oryzae* genome via NHEJ. Resulting clones vary in expression strength due to different integration genome loci. This hampers fast and comparative enzyme screening technologies in *A. oryzae*.

To generate a fast and comparative *A. oryzae* expression platform with high secretion efficiency, the three amylase genes *amyA*, *amyB* and *amyC* were deleted simultaneously using multiplex CRISPR/Cas9 editing technology resulting in the strain HGR02. The rate of obtained triple knockouts was 67 % pointing out the high efficiency of the multiplex deletion system. Further genome editing of HGR02 at the *amyC* locus was performed to enable target integration specifically at the *amyB* locus.

Target integration of an amylase expression cassette specifically into the *amyB* locus was performed using an *in vitro* CRISPR/Cas9 dual RNP system. Using this system high level secretion of the heterologously produced amylase was achieved. This amylase activity could be quantified directly from the culture supernatant without purification. The target integration specific into the *amyB* locus resulted in comparable amylase secretion level of single clones.

This new edited *Aspergillus oryzae* together with the specific target integration system can be used as a chassis for the fast and precise comparable characterization of newly identified enzyme candidates.

[1] Nemoto, Maruyama, Kitamoto; Biosci Biotechnol Biochem. 2012;76(8) DOI:10.1271/bbb.120142

---

## 058-MBTV

### Deciphering the metabolic burden in *P. putida* during heterologous protein production: Development of a genetic toolbox

\*A. S. Ortega Arbulu<sup>1</sup>, A. Kremling<sup>1</sup>, K. Pflüger-Grau<sup>1</sup>, H. Löwe<sup>1</sup>, K. Hobmeier<sup>1</sup>

<sup>1</sup>Technische Universität München, Mechanical Engineering, Garching, Germany

*Pseudomonas putida* is increasingly drawing attention as a promising host for biotechnological applications due to its highly versatile metabolism and unique resistance to endogenous and exogenous stresses. Heterologous protein overproduction, e.g. when expressing new pathways, often results in reduced cell growth and cessation of protein production as energy and metabolites, pivotal for the host's own cellular maintenance and gene expression machinery, are drained into the expression of foreign genes. In order to measure and understand the metabolic burden, we followed resource allocation during plasmid-based gene expression of eGFP, as an easily tractable model protein, in *P. putida* KT2440 and EM383. Strains engineered with the strong XylS/Pm expression system, were grown on M9-Minimal medium and glucose, and monitored spectrophotometrically. Surprisingly, KT2440 (pSEVA 438-eGFP) showed a slightly higher eGFP production rate ( $4970 \pm 200$  rlu/OD\*h) than the cell factory strain EM383 (pSEVA 438-eGFP) ( $4450 \pm 120$  rlu/OD\*h). Since *P. putida* exhibited a big gene expression leeway with no significant drop in growth rate, maltose binding protein (MBP) from *E. coli* and eGFP were genetically fused to increase the burden. *mCherry* was additionally integrated into the genome with a constitutive promoter as proxy for native gene expression. KT2440 *attTn7::lacIP-mCherry* (pSEVA 438-malEeGFP) presented a prolonged lag-phase, a substantial reduction in growth rate to  $\mu = 0.05$  h<sup>-1</sup>, accompanied by a surprisingly higher protein yield of  $Y_{P/X} = 108000 \pm 12000$  RLU/OD when compared to KT2440 *attTn7::lacIP-mCherry* (pSEVA 438-eGFP) with  $\mu = 0.18$  h<sup>-1</sup> and  $Y_{P/X} = 39000 \pm 8000$  RLU/OD. Delay of exponential growth by the fused protein might indicate metabolic stress. An increase in solubility and protein folding, as reported in literature for MBP fusions, could explain the increased eGFP yield. To rule out burden by protein toxicity, a stem-loop structure to prolong mRNA lifetime and gene expression, was constructed and is yet to be tested in shake-flask experiments. These results present a useful toolbox for the quantification of metabolic burden in the future platform organism *P. putida*.

---

## 059-MBTV

### The novel phosphatase RosC catalyzes the last unknown step of roseoflavin biosynthesis in *Streptomyces davaonensis*

\*C. Schneider<sup>1</sup>, M. Mack<sup>1</sup>

<sup>1</sup>University of Applied Sciences Mannheim, Institute for Technical Microbiology, Mannheim, Germany

The two Gram-positive soil bacteria *Streptomyces davaonensis* and *Streptomyces cinnabarinus* produce the antibiotic roseoflavin (8-demethyl-8-dimethylamino-riboflavin), which is a riboflavin analog and acts as a natural antimetabolite. Roseoflavin synthesis has been studied in *S. davaonensis* and similar enzymes are present in *S. cinnabarinus*. The first step of roseoflavin biosynthesis is the phosphorylation of riboflavin to riboflavin-5'-phosphate (RP) by the flavokinase RibC. The complex synthase RosB catalyzes the conversion from RP and glutamate to 8-demethyl-8-amino-riboflavin-5'-phosphate (AFP). Roseoflavin is synthesized by the dimethyltransferase RosA from 8-demethyl-8-amino-riboflavin (AF) via the intermediate 8-demethyl-8-methylamino-riboflavin (MAF). The RosB product AFP is not a substrate for RosA. Therefore, the roseoflavin biosynthetic pathway depends on a yet unknown phosphatase that generates AF from AFP. We report here on the identification and characterization of such a novel phosphatase. The novel enzyme was named RosC. The roseoflavin biosynthetic pathway is now fully understood. It takes four enzymes (RibC, RosB, RosC and RosA) to convert a vitamin into a potent antibiotic.



Schwarz, J., Konjik, V., Jankowitsch, F., Sandhoff, R. & Mack, M. Identification of the Key Enzyme of Roseoflavin Biosynthesis. *Angewandte Chemie (International ed. in English)* **55**, 6103–6106 (2016).

Konjik, V. *et al.* The Crystal Structure of RosB: Insights into the Reaction Mechanism of the First Member of a Family of Flavodoxin-like Enzymes. *Angewandte Chemie (International ed. in English)* **56**, 1146–1151 (2017).

## 060-MBTV

Identification of fungal limonene-3-hydroxylases for biotechnological menthol production

\*F. M. Schempp<sup>1,2</sup>, M. M. W. Etschmann<sup>1</sup>, I. Strobel<sup>1</sup>, E. Bierwirth<sup>2</sup>, J. Schrader<sup>1</sup>, M. Buchhaupt<sup>1</sup>

<sup>1</sup>DECHEMA-Forschungsinstitut, Industrial Biotechnology, Frankfurt a. M., Germany

<sup>2</sup>Goethe University Frankfurt, Faculty Biological Sciences, Frankfurt am Main, Germany

More than 30.000 tons of menthol are produced every year as flavor and fragrance compound or as medical component. So far, only extraction from plant material or chemical production is possible. An alternative approach for menthol production could be a biotechnological-chemical two-step conversion, starting from (+)-limonene, which is a side product of the citrus processing industry. The first step requires a limonene-3-hydroxylase (L3H) activity that specifically catalyzes hydroxylation of limonene at carbon atom 3. Several protein engineering strategies already attempted to create limonene-3-hydroxylases from bacterial P450s,<sup>[1–3]</sup> which can be efficiently expressed in bacterial hosts. However, their regiospecificity is rather low, if compared to the highly selective L3H enzymes from the biosynthetic pathway towards menthol in *Mentha* species.<sup>[4]</sup> The only naturally occurring limonene-3-hydroxylase activity identified in microorganisms so far, was reported for a strain of *Hormonema* black yeast in South Africa,<sup>[5]</sup> but the responsible enzyme is not yet known.

We intended to identify the underlying P450 sequences in related fungi in order to make it usable for biotechnological applications.

Apart from the described *Hormonema* strain,<sup>[5]</sup> we discovered different other fungi that can catalyze the intended reaction. In the genome of one of the strains we identified potential P450 monooxygenase-encoding genes. Using heterologous protein expression and biotransformation experiments in yeast, we were able to identify limonene-3-hydroxylases from *Aureobasidium pullulans* and *Hormonema carpetanum*.

Further characterization of both enzymes in *P. pastoris* showed their high regiospecificity, their potential for limonene-based menthol production and their additional ability to convert  $\alpha$ - and  $\beta$ -pinene into specific hydroxylation products.

<sup>[1]</sup> S. G. Bell *et al.*, *Chem. Commun.* **2001**, 7, 635–636.

<sup>[2]</sup> A. Hernandez-Ortega *et al.*, *Sci. Rep.* **2018**, 8, 14396.

<sup>[3]</sup> A. Seifert *et al.*, *ChemBioChem.* **2009**, 10, 853–861.

<sup>[4]</sup> S. Lupien *et al.*, *Arch. Biochem. Biophys.* **1999**, 368, 181–192.

<sup>[5]</sup> M. S. Van Dyk *et al.*, *J. Mol. Catal. B Enzym.* **1998**, 5, 149–154.

## 061-MBTV

Metagenomics meets Electrochemistry: harnessing the catalytic potential from the uncultured microbial majority for energy-storage

\*N. Adam<sup>1</sup>, S. Schlicht<sup>2</sup>, Y. Han<sup>3</sup>, M. Bechelany<sup>4</sup>, J. Bachmann<sup>2,5</sup>, M. Perner<sup>1</sup>

<sup>1</sup>GEOMAR Helmholtz Centre for Ocean Research Kiel, Geomicrobiology, Kiel, Germany

<sup>2</sup>Friedrich-Alexander University Erlangen, Department of Chemistry and Pharmacy, Erlangen, Germany

<sup>3</sup>Universität Hamburg, Microbiology, Hamburg, Germany

<sup>4</sup>Université de Montpellier, Institut Européen des Membranes, Montpellier, France

<sup>5</sup>Saint-Petersburg State University, Institute of Chemistry, Saint-Petersburg, Russian Federation

Hydrogen can in the future serve as an advantageous carrier of renewable energy. A prerequisite for this is that its production via water electrolysis from photovoltaic or wind power and utilization in fuel cells are realized with high energy efficiency and with abundant, non-precious electrocatalysts. In this study, we address this challenge with an enzymatic hydrogen evolution electrocatalyst as an alternative to the use of rare noble metals. The structured electrodes feature a geometrically defined large specific surface area, to allow low overpotentials to be used and thereby high energy efficiencies to be achieved. The unprecedented novelty of this study is the use of hydrogen converting enzymes from unculturable microorganisms, which are harnessed by a recently developed activity-based screen of metagenomic libraries. The respective libraries were constructed from deep-sea hydrothermal vent systems hallmarked by dynamic hydrogen and oxygen concentrations as well as steep thermal gradients. One particularly efficient hydrogen converting recombinant enzyme was chosen for further applications. When dispersed on a TiO<sub>2</sub>-coated polyacrylonitrile fiber electrode, it exhibited a 3-fold higher activity than the hydrogenase of the host in which it was heterologously expressed. Furthermore, it is characterized by an unusually high stability. These results highlight the tremendous potential of enzymes derived from the uncultured microbial majority for applications in energy conversion and storage technologies.

## 062-MBTV

Biophotovoltaics using Cyanobacteria

B. Lai<sup>1</sup>, H. Schneider<sup>1</sup>, J. Tschörtner<sup>1</sup>, \*J. Krömer<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Solar Materials, Leipzig, Germany

Biophotovoltaics is a new discipline in microbial fuel cell research. The basic idea is the conversion of light energy into electrical energy using photosynthetic microorganisms. Oxygenic Cyanobacteria are the microbes most widely applied in biophotovoltaics research. The microbes will use their photosynthetic apparatus and the incoming light to split the water molecule. The generated protons and electrons are harvested using a bioelectrochemical system. The key challenge is the extraction of electrons from the microbial electron transport chain into a solid state anode. On the cathode a corresponding electrochemical counter reaction will consume the protons and electrons, e.g. through the oxygen reduction to water, or hydrogen formation.

Here we present a system that generates light dependent current output and H<sub>2</sub> formation by *Synechocystis* PCC6803,

a model organism for oxygenic cyanobacteria. We have employed a mediated electron transfer process using potassium ferricyanide (K<sub>3</sub>Fe(CN)<sub>6</sub>) as electron shuttle between the cells and the anode surface. Since the current outputs of such a system are quite low, reproducibility was difficult to achieve. Careful optimization of the reactor set-up as well as a systematic screening of electrochemical interference from media components was necessary and subsequent media optimization was conducted. The resulting optimized system allowed measurement of current output and could be used to differentiate between electrons derived from light-driven water oxidation and those generated by metabolism of storage carbohydrates in darkness. Using measurement of intracellular glycogen pools, energy charge of the adenosin pool and degree of reduction of the nicotinamid pools allowed to characterize the process in more detail.

The results obtained here are currently used in combination with metabolic engineering to improve current densities. This not only provides deeper understanding of the electron flows in cyanobacteria and thus improves our understanding of photosynthesis but this also lays the basis for energy efficient light-driven production of green hydrogen without the generation of explosive oxyhydrogen.

### 063-MSRV

Formation of reactive oxygen species in an ATP-Synthase deficient mutant of *Salmonella* Typhimurium

\*S. Braetz<sup>1</sup>, P. Schwerk<sup>1</sup>, A. Thompson<sup>1,2</sup>, K. Tedin<sup>1</sup>, M. Fulde<sup>1</sup>

<sup>1</sup>Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany

<sup>2</sup>Microbial Therapeutic Solutions, Norwich, United Kingdom

Bacteria with an increased tolerance against antibiotics without acquiring genetic resistance are termed persisters. When re-cultured in the absence of antibiotics, persisters show the same drug susceptibility as the original population. Currently, low metabolic activity, reduced ATP concentrations, as well as the activation of toxin/antitoxin modules are believed to be involved in persister formation [1-3].

Here, we investigated persister cell formation in an *atp* operon deletion strain of *Salmonella* Typhimurium by an exposition of 4-fold the MIC of ciprofloxacin (CIP). We examined the involvement of reactive oxygen species (ROS) in killing and performed persister assays under ROS diminishing conditions. In addition, we determined the metabolic activity, NADH content, and persistence in strains of *S. Typhimurium* harboring mutations in metabolic genes involved in pathways associated with persister formation through endogenous ROS production.

Despite reduced ATP pools, the *atp* operon deletion mutant was found to be more susceptible to CIP, although previous studies demonstrated that reduced ATP concentrations result in increased persister cell formation [2,3]. We show that deletion of the *fre* gene encoding flavin reductase in the *atp* deletion background partially restored persister cell formation to wild-type levels, as did ROS diminishing conditions. Furthermore, we observed higher levels of NADH, increased metabolic activity and elevated hydroxyl radical formation in the *atp* operon mutant.

Our results suggest that the lack of the ATP Synthase increases the metabolic activity through increased glycolysis, resulting in the accumulation of NADH/NADPH, which in turn drives the flavin reductase to elevated side reactions

reducing ferric iron to ferrous iron, thus promoting hydroxyl radical formation via the Fenton reaction.

1. Germain, E., et al., *Molecular mechanism of bacterial persistence by HipA*. Mol Cell, 2013. **52**(2): p. 248-54.
2. Shan, Y., et al., *ATP-Dependent Persister Formation in Escherichia coli*. MBio, 2017. **8**(1).
3. Conlon, B.P., et al., *Persister formation in Staphylococcus aureus is associated with ATP depletion*. Nat Microbiol, 2016. **1**: p. 16051.

### 064-MSRV

Responses to reactive oxygen and chlorine species in uropathogenic *Escherichia coli*

\*J. U. Dahl<sup>1</sup>, G. Anderson<sup>1</sup>, S. Sultana<sup>1</sup>, K. P. Hoffmann<sup>1,2</sup>, M. Hall<sup>1</sup>, K. Ledoux<sup>1</sup>

<sup>1</sup>Illinois State University, School of Biological Sciences, Normal, United States

<sup>2</sup>DAAD RISE FELLOW, Technical University Braunschweig, Braunschweig, Germany

Urinary tract infections (UTIs) are among the most common bacterial infections and mainly caused by uropathogenic *E. coli* (UPEC). UPEC differ significantly from commensal intestinal *E. coli* by their considerably larger genome, which enables them to survive and persist in the urinary tract. During UTI, UPEC experience an increased number of neutrophils that produce high levels of reactive oxygen and chlorine species (RO/CS), among which is hypochlorous acid (HOCl), the active ingredient of household bleach and the most commonly used disinfectant in medical, industrial and domestic settings. Due to the increased HOCl levels present in the bladder, we hypothesize that UPEC strains need particularly efficient HOCl-defense systems in order to successfully colonize the urinary tract. Therefore, our goal is to comprehensively understand the cause and effects of RO/CS stress in UPEC and identify specific defense strategies used to limit their damage.

Our growth and survival data suggest that UPEC strains are substantially more HOCl-resistant than the commensal *E. coli* strain MG1655. This is particularly significant since MG1655 is considered to be one of the most robust *E. coli* lab strains known. Moreover, the HOCl resistance of enteropathogenic *E. coli* is similar to MG1655 and significantly lower than UPEC. Contrary, no difference in sensitivity among UPEC, EPEC and commensal *E. coli* was observed in response to the neutrophilic oxidant peroxide excluding the possibility that UPEC resistance is non-specific to RO/CS in general. We found that expression the HOCl-specific *rciA* gene was significantly more induced in HOCl-treated MG1655 compared to CFT073, supporting the idea that CFT073 employs additional defense systems against HOCl stress. Our transcriptomic data reveal the upregulation of UPEC-specific transcriptional regulators. Based on our observations, we propose that UPEC strains have acquired increased resistance towards HOCl, which likely contribute to UPEC pathogenesis. Identifying, characterizing and targeting HOCl-specific defense systems has the potential to increase the HOCl sensitivity in UPEC and mitigate HOCl-caused damage.

### 065-MSRV

TisB-dependent persister cells fail to induce an SOS response due to impeded protein biosynthesis

\*D. Edelmann<sup>1</sup>, N. E. Schmid<sup>1</sup>, B. Berghoff<sup>1</sup>

<sup>1</sup>University of Giessen, Institute for Microbiology and Molecular Biology, Gießen, Germany

Bacterial persister cells are considered to be one of the major contributing factors of recalcitrant infections. Several different routes into a persister state have been identified until today, but reduced growth and cellular activity appear to be common traits. Previous studies have shown that the depolarizing toxin TisB contributes to persister formation in *Escherichia coli* during a ciprofloxacin treatment and that mild TisB overexpression renders *E. coli* extraordinarily persistent against antibiotic treatments [1,2]. New evidence suggests that TisB affects both cellular activity and protein biosynthesis. Microscopy and fluorescent protein production assays revealed that wild-type cells show massive cell elongation and protein biosynthesis even after 5 hours of ciprofloxacin treatment. In contrast, a regulatory TisB mutant with an increased population of TisB-dependent persisters stops both processes within minutes when exposed to ciprofloxacin. Reporter assays with the SOS response gene *sulA* as a marker for DNA damage indicate that TisB-dependent persisters effectively evade the impact of this particular antibiotic, likely due to inactivation of target molecules. However, we find the regulatory mutant maladapted when DNA damage cannot be circumvented, e.g. during a treatment with the DNA-crosslinking agent mitomycin C, which does not require a target to be active. We speculate that the inability of the regulatory mutant to mount an effective SOS response is explained by a global reduction of protein biosynthesis. The lack of an SOS response proves to be a severe disadvantage during a mitomycin C treatment. Hence, we observe a drastically reduced survival rate for a strain highly persistent against other antibiotic classes. We conclude that TisB-dependent persistence is yet another example of an antibiotic-specific endurance mechanism in bacteria.

[1] Dörr *et al. PLoS Biol.* **8**, 29–35 (2010).

[2] Berghoff *et al. Mol. Microbiol.* **103**, 1020–1033 (2017).

## 066-MSRV

### Investigation of the post-transcriptional regulation of the emerging cancer-associated bacterium *Fusobacterium nucleatum*

\*F. Ponath<sup>1</sup>, F. Faber<sup>2</sup>, J. Vogel<sup>1,3</sup>

<sup>1</sup>Helmholtz Institute for RNA-based Infection Research (HIRI), RNA Biology of Bacterial Infections (RABI), Würzburg, Germany

<sup>2</sup>Julius-Maximilians-University of Würzburg, Research Center for Infectious Diseases (ZINF), Würzburg, Germany

<sup>3</sup>Julius-Maximilians-University of Würzburg, Institute for Molecular Infection Biology (IMIB), Würzburg, Germany

#### Question:

The anaerobic gram-negative bacterium *Fusobacterium nucleatum* (FN) is increasingly gaining attention due to its role in diseases outside of its natural habitat. Recent studies found a significant association of FN with colorectal cancer (CRC) or adenoma tissue for which its increased presence correlates with worsened patient prognosis and resistance to common chemotherapeutic. Despite this, only few genes have been characterized and even less is known about the post-transcriptional regulation in FN and its possible connection to CRC. To remedy this, we take an RNA-centric approach of understanding the post-transcriptional regulation of FN by investigating key regulators such as small non-coding RNAs (sRNAs) and their function in its life style.

#### Methods:

We analyzed the primary transcriptome using differential RNA-seq (dRNA-seq) and total RNA-seq during growth

stages and exposure to gut-associated conditions. Additional standard methods were used to investigate the function of newly identified sRNAs.

#### Results:

dRNA-seq allowed the precise identification of transcriptional start sites and promoter regions for FN while also enabled the discovery of novel sRNAs. Exposing FN to different gut-associated conditions allowed the characterization of transcriptional units involved in its response to external stress factors. These factors also led to differential expression of multiple sRNAs, indicating an important role in coping with these environmental changes. In order to identify targets of promising sRNA candidates, we genetically manipulated the sRNAs and characterized them regarding global transcriptomic changes and the impact on growth or biofilm formation.

#### Conclusion:

Here we report the detailed primary transcriptome of FN achieved by dRNA-seq and additionally allowed the discovery of novel sRNAs in FN. We complemented this by analyzing the transcriptomic response to different gut-associated conditions. Combined, our results on the global transcriptomic changes and the characterization of the sRNAs enabled us to improve our understanding of the regulatory role of the sRNAs and shed light on the CRC-associated pathogen's physiology.

## 067-MSRV

### Hfq-dependent and -independent mechanisms of RNA-based regulation in *Caulobacter crescentus*

\*K. Fröhlich<sup>1</sup>

<sup>1</sup>University of Jena, Microbiology, Jena, Germany

The water-dwelling alpha-proteobacterium *Caulobacter crescentus* is able to thrive in nutrient-poor environments. Its lifestyle is controlled by an intricate network of transcriptional activation and repression mechanisms, differential phosphorylation of two-component system regulatory proteins, and proteolysis of regulatory and structural proteins. However, regulation at the post-transcriptional level - hence the contribution of RNA-mediated regulation - which has been identified as a central component of gene expression regulation in other bacteria is only about to be explored.

Bacterial small RNAs (sRNAs) are a heterogeneous group of post-transcriptional regulators that often act at the heart of large networks. Most sRNAs characterized today engage in direct base-pairing interactions to modulate translation and/or stability of target mRNAs. In many cases, the ubiquitous RNA chaperone, Hfq, contributes to the establishment of functional interactions. We have recently solved the X-ray crystal structure of the RNA chaperone protein, and determined its principal ability to promote RNA annealing. Here we report on the identification of sRNAs associated with Hfq in *C. crescentus*, and for the first time the characterization of Hfq-dependent post-transcriptional regulation in this organism. Using transcriptomics we have uncovered a large set of targets controlled by one Hfq-bound and have validated its activity both *in vivo* and *in vitro*.

In addition, we characterized a novel sRNA, ChvR, controlled by the conserved two-component system ChvI-ChvG. ChvR is expressed in response to DNA damage, low pH, and growth in minimal medium. We identified the mRNA of the

TonB-dependent receptor ChvT as the sole target of ChvR. Genetic and biochemical analyses showed that ChvR represses ChvT at the post-transcriptional level through the formation of two distinct base-pairing interactions. Finally, we show that ChvR-controlled repression of *chvT* is independent of Hfq, and therefore distinct from previously reported mechanisms employed by prototypical bacterial sRNAs.

---

## 068-MSRV

### The destructive side of gene regulation - or the impact of RNA degradation in stress responses

L. Youssar<sup>1</sup>, G. Kostova<sup>1</sup>, W. R. Hess<sup>1</sup>, \*J. Georg<sup>2</sup>

<sup>1</sup>University of Freiburg, Genetics and Experimental Bioinformatics, Freiburg i. Br., Germany

<sup>2</sup>University of Freiburg, Computational RNA analysis (Junior Group), Freiburg i. Br., Germany

Gene regulation is often investigated from a synthesis-centric angle. In this view, specific sigma or transcription factors activate or repress the transcription of specific genes in response to certain signals or growth phases. However, there is no equilibrium or turning off of gene expression without RNA degradation, the destructive side of the coin.

Furthermore, it is well established that RNA levels can be also controlled by post-transcriptional regulation, e.g. through small regulatory RNAs (sRNAs). Nevertheless, a standard gene expression analysis only shows the differences in the equilibrium RNA levels, but cannot disentangle if these are due to changes in the RNA synthesis (transcriptional) or changes in the degradation rates (post-transcriptional).

Here, we investigate both the differences in gene expression and the differences in RNA degradation rates at standard and iron stress conditions using the cyanobacterium *Synechocystis* sp. PCC 6803 as a model. The analysis allowed to estimate the relative shares of transcriptional and post-transcriptional regulation in the observed total regulation. As an example, the regulon controlled by the iron stress sRNA *IsaR1*<sup>1</sup> became distinctively visible. Other genes did not show a differential expression but the degradation rate was clearly changed, indicating a converse transcriptional and post-transcriptional regulation, which leveled out each other.

Furthermore, we detected different modes of regulation, e.g. degradation before the mRNA is fully synthesized or degradation not until completion of transcription. Other degradation patterns did not fit to any of these modes and indicate interesting alternative modes of degradation.

Finally, the dataset helped us to disentangle the complex transcriptional organization of the *Synechocystis* genome, with multiple instances of nested and alternative transcriptional start site as well as mRNA fragments of different stabilities, which together determine the fate of an individual transcript variant.

1. Georg J., et al. 2017. "Acclimation of Oxygenic Photosynthesis to Iron Starvation Is Controlled by the sRNA *IsaR1*." *Current Biology*: CB 27 (10): 1425–36.e7.

---

## 069-MSRV

### Analysis of ultrastructure of akinetes and the role of *hetM* in heterocyst and akinete envelope formation

\*R. Garg<sup>1</sup>, I. Maldener<sup>1</sup>

<sup>1</sup>Interfaculty Institute of Microbiology and Infection Medicine, Organismic Interactions, University of Tübingen, Tübingen, Germany

The filamentous cyanobacterium *Anabaena variabilis* ATCC 29413 can differentiate heterocysts and akinetes to survive under different stress conditions. Under nitrogen limited condition, heterocysts provide the filament with nitrogen by fixing N<sub>2</sub> [1]. Akinetes are spore-like dormant cells, which allow survival during adverse growth conditions. They differ from vegetative cells by their cellular structure, composition, and morphology and exhibit low metabolic activities [2]. They can germinate to produce new filaments under suitable conditions and are resistant to cold and desiccation [1].

Our research focuses on elucidating the mechanism of the morphological changes during akinetes formation and germination. Using scanning electron microscopy, we found that the mature akinetes have a wrinkled envelope structure and during germination, the cell size increases, surface smoothens, and the short filament emerges. Focused Ion Beam (FIB)/SEM images indicated the presence of cytoplasmic granules in immature akinetes and different layers consisting of exopolysaccharide and glycolipid in akinetes envelope. The akinetes envelope was found to be required for maintenance of structural integrity and helps to withstand the osmotic stress.

Formation or deposition of the heterocyst glycolipid layer required the specific genes cluster including the gene *hetM* (*Ava\_2595*). Since akinetes may have been the ancestors of heterocysts and share some common regulatory pathways for differentiation [1], we mutated the gene *hetM* and observed delayed heterocyst and akinete differentiation. Heterocyst formation was aberrant and the mutant was not able to grow without combined nitrogen source. Bodipy staining showed the absence of glycolipid layer in heterocysts and akinetes envelope in the mutant, which was further confirmed by electron microscope analysis and thin layer chromatography.

## References

Maldener I, et al. (2014) Cellular differentiation in filamentous cyanobacteria. *The Cell Biology of Cyanobacteria*: 263-291.

Perez R, et al. (2016) Clear differences in metabolic and morphological adaptations of akinetes of two Nostocales living in different habitats. *Microbiology*. 162(2):214-23.

---

## 070-MSRV

### Quantification of outer membrane vesicles: a potential tool to compare response in *Pseudomonas putida* KT2440 to stress caused by alkanols.

\*C. Eberlein<sup>1</sup>, S. Starke<sup>1</sup>, H. J. Heipieper<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Biotechnology, Leipzig, Germany

The bacterial release of outer membrane vesicles (OMVs) is an important physiological mechanism of Gram-negative bacteria playing numerous key roles. One function of the release of OMVs is related to an increase in surface hydrophobicity. This phenomenon initiates biofilm formation, making bacteria more tolerant to environmental stressors. Recently, it was qualitatively shown for *Pseudomonas putida* that vesicle formation plays a crucial role in multiple stress responses. Yet, no quantification of OMVs for certain stress scenarios has been conducted. In this study, it is shown that the quantification of OMVs can serve as a simple and feasible tool, which allows a comparison of vesicle yields for different experimental setups, cell densities, and environmental stressors. Moreover, the obtained results provide insight to the underlying mechanism of vesicle formation as it was observed that *n*-alkanols, with a chain

length of C7 and longer, caused a distinct and steep increase in vesiculation (12-19-fold), compared to shorter chain n-alkanols (2-4-fold increase).

---

## 071-SCMCV

Phenotypic growth heterogeneity of *C. glutamicum* under dynamic growth limiting environmental conditions

S. Täuber<sup>1</sup>, C. Golze<sup>1</sup>, H. Steinhoff<sup>1</sup>, \*A. Grünberger<sup>1</sup>

<sup>1</sup>Bielefeld University, Faculty of Technology - Multiscale Bioengineering, Bielefeld, Germany

## Question

Microorganisms can perform multiple metabolic functions, both in natural habitats as well as biotechnological production processes. The rates at which different activities are performed can vary between individual cells and the surrounding environment [1]. The molecular mechanisms with influence of metabolic heterogeneity have attracted significant research interest in the last years [2], but the difference in the environmental conditions which can affect metabolic heterogeneity have received little research attention. To investigate this, novel analytical methods and workflows need to be established.

## Objectives and Methods

In this contribution, we introduce a microfluidic single-cell workflow for the cultivation of microbial cells at dynamic environmental conditions [3]. This system allows to oscillate between different environmental input parameters e.g. pH values or carbon sources.

## Results

In a proof of concept study, we cultivated *Corynebacterium glutamicum* at oscillating medium conditions, switching between medium rich (complex media) and a buffer (no nutrients) with different oscillation frequencies ranging from hours to minutes intervals. We could show that the oscillating frequency has not only an impact on the overall growth rate, but significantly affects cell morphology. Especially for high oscillation frequencies (10 minutes intervals), cell physiology seems so be significantly affected [3]. Latest results, hypothesis regarding the observed growth pattern and potential application will be shown.

## Conclusion

Our results show that the concept of dynamic microfluidic single-cell cultivation has a high potential to investigate cellular physiology at environmental perturbations. This paves the way for an improved understanding of how environmental conditions shape metabolic heterogeneity.

[1] Pearman, P. B., et al., **2008**, *Trends in ecology & evolution*, **23**:149–158.

[2] Ackermann, M., et al, **2015**, *Nature Reviews Microbiology* **13**:497-508

[3] Täuber, S., et al., **2020**, manuscript in preparation.

---

## 072-SCMCV

## BiofilmQ, a software tool for quantitative image analysis of microbial biofilm communities

R. Hartmann<sup>1</sup>, \*H. Jeckel<sup>1,2</sup>, E. Jelli<sup>1,2</sup>, P. K. Singh<sup>1</sup>, S. Vaidya<sup>1</sup>, M. Bayer<sup>1</sup>, L. Vidakovic<sup>1</sup>, F. Díaz-Pascual<sup>1</sup>, J. C. N. Fong<sup>3</sup>, D. Anna<sup>4</sup>, O. Besharova<sup>1</sup>, C. Nadell<sup>5</sup>, V. Sourjik<sup>1,6</sup>, † T. Kocács<sup>4</sup>, F. H. Yildiz<sup>3</sup>, K. Drescher<sup>1,2,6</sup>

<sup>1</sup>Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany

<sup>2</sup>Philipps University Marburg, Department of Physics, Marburg, Germany

<sup>3</sup>University of California, Department of Microbiology and Environmental Toxicology, Santa Cruz, CA, United States

<sup>4</sup>Technical University of Denmark, Department of Biotechnology and Biomedicine, Kongens Lyngby, Denmark

<sup>5</sup>Dartmouth College, Department of Biological Sciences, Hanover, NH, United States

<sup>6</sup>Zentrum für Synthetische Mikrobiologie, Marburg, Germany

Biofilms are now considered to be the most abundant form of microbial life on Earth, playing critical roles in biogeochemical cycles, agriculture, and health care. Phenotypic and genotypic variations in biofilms generally occur in three-dimensional space and time, and biofilms are therefore often investigated using microscopy. However, the quantitative analysis of microscopy images presents a key obstacle in phenotyping biofilm communities and single-cell heterogeneity inside biofilms. Here, we present BiofilmQ, a comprehensive image cytometry software tool for the automated high-throughput quantification and visualization of 3D and 2D community properties in space and time. Using BiofilmQ does not require prior knowledge of programming or image processing and provides a user-friendly graphical user interface, resulting in editable publication-quality figures. BiofilmQ is designed for handling fluorescence images of any spatially structured microbial community and growth geometry, including microscopic, mesoscopic, macroscopic colonies and aggregates, as well as bacterial biofilms in the context of eukaryotic hosts.

---

## 073-SCMCV

### Characterizing host-pathogen interaction dynamics in single cells using scSLAM-seq

\*E. Saliba<sup>1</sup>, S. Helaine<sup>2</sup>, P. Hill<sup>2</sup>, C. Toussaint<sup>1</sup>

<sup>1</sup>Helmholtz Institute for RNA-based Infection Research (HIRI), Würzburg, Germany

<sup>2</sup>Harvard Medical School, Department of Microbiology, Boston, United States

During the *ex vivo* macrophage infection by the intracellular pathogen *Salmonella enterica* serovar Typhimurium, the host cell undergoes a two-step transition (i) from a quiescent state to a pro-inflammatory state (so-called M1) after invasion, (ii) from a pro-inflammatory state to an anti-inflammatory state (so-called M2) when the bacteria starts to replicate. These complex sequences of host cell fate changes happen in few hours and are fundamental to allow the bacteria to deploy its virulence program and establish an intracellular home allowing the pathogen to replicate or niche as a persister (Saliba et al, 2016; Stapels et al., 2018). How cells are able to transition from one state to the other remains an open question. Here we introduce a time-resolved single-cell RNA-seq method based on the measurement of nascent RNAs that allows to capture transcriptomics changes after infections.

Current transcriptomics methods have captured snapshots of these states but so far we have a very little understanding of *trajectories* meaning what transcription factors govern the phenotypic changes. Measurement of "fast" gene expression changes upon infections have been hindered by the presence of pre-existing RNAs and current single-cell RNA-seq have no true temporal dimensions. We propose here to use the measurement of nascent RNAs to record the cellular



history along the infection process. To this end, we used a method called scSLAM-seq that we recently introduced (Erhard et al 2019) based on nucleotide labeling, biochemical nucleotide conversion and single-cell RNA-seq. scSLAM-seq measures simultaneously total and new RNAs revealing the order of transcriptional programs that takes place upon *Salmonella* infection. scSLAM-seq is ready to be used for other bacterial infection models and to monitor drug response.

Erhard F et al, *scSLAM-seq reveals core features of transcription dynamics in single cells* (2019) **Nature**

Saliba AE et al, *Single-cell RNA-seq ties macrophage polarization to growth rate of intracellular *Salmonella** (2016) **Nature Microbiology**

Stapels DA et al, *Salmonella persists actively undermine host immune defences during antibiotic treatment* (2018) **Science**

---

## 074-SCMCV

Antibiotics impact on natural microbial communities - immediate effects on single cell activities and microbial diversity

\*F. Calabrese<sup>1</sup>, S. C. Chen<sup>1</sup>, H. Stryhanyuk<sup>1</sup>, F. Musat<sup>1</sup>, M. Brauns<sup>2</sup>, H. H. Richnow<sup>1</sup>, N. Musat<sup>1</sup>

<sup>1</sup>Helmholtz-Zentrum für Umweltforschung GmbH - UFZ, Isotope Biogeochemistry Department, Leipzig, Germany

<sup>2</sup>UFZ, River Ecology, Magdeburg, Germany

In the past decades, the use of antibiotics increased due to their application for treating human infections, preventing diseases in agriculture and fostering livestock growth in farms. This massive use resulted in environmental contamination with the consequent development and spread of antibiotic resistance, but also in antibiotic absorption in crops via manure as well as their run-off into streams, rivers and lakes. The currently unknown effects of antibiotics on the ecology and the physiology of natural microbial communities may have irreversible long-term impact on the biochemical elements cycling and the ecosystem functioning. Especially important are the effects caused by antibiotics on natural aquatic communities with direct impact on the water quality.

In the present study we used stable isotope probing in combination with nanoscale secondary ion mass spectrometry (SIP-nanoSIMS) to analyze the effects of two commonly used antibiotics Amoxicillin and Ciprofloxacin on single-cell microbial activities, measured as uptake of carbon and nitrogen, and 16SrRNA amplicon sequencing for monitoring the species composition and structure of two aquatic communities. River water samples were collected and incubated for 24 hours at in situ conditions in the presence of labelled carbon and nitrogen (13C-acetate and 15N-ammonium) at different antibiotic concentrations (1 µg/L, close to concentrations measured in water bodies; 1 mg/L, simulating potential spills or run-offs).

Our results show that single-cell C- and N-uptakes were strongly influenced by the antibiotics addition: the higher the concentration the bigger the gap between highly and lowly metabolically active cells. Single-cell activity measurements were supported by drastic reductions in microbial diversity or changes in species abundance, suggesting a strong selection pressure on the microbial community for both applied antibiotics at both concentrations.

We conclude that antibiotics presence has immediate effects on microbial activity, diversity and structure of natural microbial communities, already detectable after short term exposure.

---

## 075-SCMCV

Using Click-CLEM to reveal the mechanism of the bactericidal effect of azido modified ceramides against *Neisseria meningitidis*

\*S. Peters<sup>1</sup>, L. Kaiser<sup>1,2,3</sup>, J. Fink<sup>2</sup>, J. Schlegel<sup>4</sup>, C. Stigloher<sup>2,3</sup>, M. Sauer<sup>4</sup>, J. Seibel<sup>2</sup>, A. Schubert-Unkmeir<sup>1</sup>

<sup>1</sup>Julius-Maximilians-University of Würzburg, Institut für Hygiene und Mikrobiologie, Würzburg, Germany

<sup>2</sup>Julius-Maximilians-University of Würzburg, Institute of Organic Chemistry, Würzburg, Germany

<sup>3</sup>Julius-Maximilians-University of Würzburg, Division of Electron Microscopy, Würzburg, Germany

<sup>4</sup>Julius-Maximilians-University of Würzburg, Department of Biotechnology and Biophysics, Würzburg, Germany

Introduction: Antibiotic-resistant bacteria represent a major problem worldwide, making the identification of novel anti-microbial compounds a priority to achieve. Sphingolipids, including ceramides, are a diverse group of structurally related lipids. The sphingosine backbone alone shows antibacterial activity against a broad range of pathogenic microorganisms, including Gram-positive and negative bacteria and fungi. In addition, recent studies show a growth inhibitory effect of ceramide analogs against *Chlamydia trachomatis* and *Neisseria meningitidis*.

Aim: In this study, we used a combination of click chemistry and correlative light and electron microscopy (CLEM) to analyze the mechanism of the bactericidal effect of azido-functionalized ceramides ( $\omega$ -C<sub>6</sub>-Ceramide) against the human pathogen *N. meningitidis* (*Nm*) by observation of ultrastructural damage and the localization of  $\omega$ -C<sub>6</sub>-Ceramide in *Nm* after treatment.

Methods: We first estimated the minimal inhibitory concentration and minimal bactericidal concentration of the  $\omega$ -C<sub>6</sub>-Ceramide. Next, we used scanning/transmission electron microscopy (SEM/TEM) to observe ultrastructure alterations in *Nm* and utilized CLEM to observe the localization of ceramides in *Nm*. To visualize the  $\omega$ -C<sub>6</sub>-Ceramide by CLEM we took advantage of click chemistry in which the azide of the ceramide reacts with a dye coupled to an alkyne by strain promoted alkyne-azide cycloaddition.

Results: We observed ultrastructural damage of the bacterial outer membrane after incubation with the  $\omega$ -C<sub>6</sub>-Ceramide as determined by SEM/TEM. By CLEM, we showed that the ceramide integrated into the bacterial membrane, which may explain the damage shown. Additionally, we found an accumulation of the ceramides in the bacterial cytosol.

Conclusion: CLEM, combined with click chemistry, offers a powerful tool to study the effects of antimicrobial compounds against pathogenic bacteria. Besides the easy handling, this approach has the advantage of avoiding artifacts because it precludes the need for genetic tagging or antibody labeling.

---

## 076-SCMCV

Role of sRNA 5'-phosphorylation state for target RNA regulation in *E. coli*

\*A. Schilder<sup>1</sup>, B. Görke<sup>1</sup>

<sup>1</sup>Max Perutz Labs, Vienna Biocenter (VBC), Wien, Austria

In *Enterobacteriaceae* RNA degradation is initiated by endoribonuclease RNase E. The 5'-end-dependent mechanism of RNase E cleavage relies on the conversion of 5'-triphosphate (5"-PPP) groups of primary transcripts to 5"-monophosphate (5"-P) groups by the pyrophosphohydrolase RppH. 5"-Terminal-monophosphates can allosterically activate RNase E by contacting the catalytic domain (Bandyra *et al.*, 2018, *Mol Cell* 72:1-11). Some base-pairing small RNAs (sRNAs) trigger degradation of their target RNAs by recruiting RNase E. In a previous *in vitro* study it was shown that the regulatory potential of MicC sRNA towards its target mRNA is higher when MicC is present in the 5"-P form as compared to the 5"-PPP form (Bandyra *et al.*, 2012, *Mol Cell* 47:943-953). Thus, an RNA silencing pathway was suggested, where 5"-P sRNAs accelerate target turnover by allosteric activation of RNase E. Moreover, recently an RNA aptamer was discovered that provides so far the only tool to generate 5"-P RNA species *in vivo* on demand (Göpel *et al.*, 2016, *NAR* 44:824-837).

In the current study, the aptamer was used to establish a conditional cleavage system allowing the release of 5"-P sRNAs to study their impact on target RNA degradation *in vivo*. sRNAs that are generated via primary transcription (e.g. MicC, RyhB) are highly unstable in their monophosphorylated forms *in vivo*. Accordingly, the 5"-PPP forms exerted a stronger or comparable regulatory effect on their targets as compared to the 5"-P variants. In contrast, the 5"-phosphorylation state has no impact on stability of sRNAs that are naturally monophosphorylated (e.g. CpxQ, SroC), as they are generated via processing. The latter sRNA class yielded comparable effects on its target RNAs, regardless of their 5"-phosphorylation status and the presence of RppH. In some cases, target degradation was even slightly more efficient in presence of the 5"-PPP sRNA as in presence of the respective 5"-P sRNA variant. Based on our results, it appears that 5"-P sRNA-stimulated target degradation by RNase E does not represent a mechanism operating *in vivo*.

## 077-SCMCV

### A gap junction analog confers cell-cell communication in multicellular cyanobacteria

A. K. Kieninger<sup>1</sup>, G. Weiss<sup>2</sup>, M. Pilhofer<sup>2</sup>, R. Garg<sup>1</sup>, K. Forchhammer<sup>1</sup>, \*I. Maldener<sup>1</sup>

<sup>1</sup>University of Tübingen, IMIT Organisms Interactions, Tübingen, Germany

<sup>2</sup>ETH, Department of Biology, Institute of Molecular Biology & Biophysics, Zürich, Switzerland

Cells of the multicellular cyanobacterium *Nostoc* (*Anabaena*) PCC 7120 communicate along the filament using direct cell-cell connections, the septal junctions (SJ)<sup>1</sup>. These proteinaceous structures traverse the septal peptidoglycan thru nanopores<sup>2</sup> and allow the exchange of small molecules between the cytoplasm of adjacent cells<sup>1</sup>.

We recently resolved the *in situ* architecture of the SJs and showed that they are gated communication channels, resembling ancient gap junction analogs<sup>3</sup>. The SJ are composed of a tube traversing the nanopores, ending at each cytoplasmic membrane in a plug structure, which is covered in the cytosolic site by a cap module. By using different inhibitors or applying natural stress conditions we showed that the cap changes its conformation ceasing communication. This showed that the SJs can control the molecule transport between the cells. We also identified one protein as component of the plug, the septal protein FraD. Mutants lacking FraD are not able to close their SJ upon stress, showing that this structural module is required for the gating of the SJs. We currently investigate the gating

mechanism and the cell-cell communication between heterocysts, specialized in nitrogen fixation, and their neighboring vegetative cells.

1 Flores *et al.* Life 2019, **9**

2 Lehner, *et al.* (2013), *FASEB J*, **27**

3 Weiss, Kieninger *et al.* (2019), *Cell*, **178**

## 078-SCMCV

### Regulation of polar flagellar assembly in *Shewanella putrefaciens*

\*M. Schwan<sup>1</sup>, D. Mrusek<sup>2</sup>, V. Blagotinsek<sup>2</sup>, T. Glatter<sup>3</sup>, A. Khaledi<sup>4</sup>, S. Häußler<sup>4</sup>, K. Papenfort<sup>5</sup>, G. Bange<sup>2</sup>, K. Thormann<sup>1</sup>

<sup>1</sup>Justus-Liebig Universität Gießen, Institut für Mikro- und Molekularbiologie, Gießen, Germany

<sup>2</sup>Philipps Universität Marburg, LOEWE Zentrum für Synthetische Mikrobiologie, Marburg, Germany

<sup>3</sup>Max Planck Institute for Terrestrial Microbiology, Massenspektrometrie und Proteomics, Marburg, Germany

<sup>4</sup>Helmholtz Zentrum für Infektionsforschung, Molekulare Bakteriologie, Braunschweig, Germany

<sup>5</sup>Friedrich-Schiller Universität, Institut für Mikrobiologie, Jena, Germany

Flagella are organelles of locomotion and multiprotein complexes whose assembly and positioning requires complex spatiotemporal control. Generally, flagellar assembly is thought to be controlled in several transcriptional tiers, which is mediated through several master regulators. Here, we have studied the regulation of flagellar genes in polarly flagellated gammaproteobacteria by the regulators FlrA, RpoN ( $\sigma$ 54) and FliA ( $\sigma$ 28) in *Shewanella putrefaciens* CN-32 as a general model-organism. We show that the transcriptional order of events also exist in *Shewanella* but is not reflected at the protein level.

The major regulators, FlrA and RpoN, activate the transcription of the basal body building blocks and flagellar export gate. The sigma factor FliA regulates the late flagella components. Notably, only a few of the early flagella proteins appear to be regulated at the protein level while most are present at normal levels also in the absence of the master regulators. The data indicates that strict control at both the transcriptional and protein level only occurs for key components for initiation of flagellar assembly (such as some early C-ring components), motor activation (such as MotY) and for highly abundant proteins, such as the outside structures (such as hook proteins and flagellins).

In addition, we investigated the regulation of the MinD-like ATPase FlhG, which determines the number of flagella. A negative feedback of the FlrA-dependent gene expression is triggered by the interaction of FlhG with the HTH-domain of FlrA. These findings provide new insights into coupling of transcriptional regulation and assembly of polar bacterial flagella.

## 079-RCV

### Controversies in bacterial taxonomy: The example of the genus *Borrelia*

\*G. Margos<sup>1</sup>, V. Fingerle<sup>1</sup>, A. Sing<sup>1</sup>, S. Cutler<sup>1</sup>, A. Gofton<sup>1</sup>, B. Stevenson<sup>1</sup>, A. Estrada-Peña<sup>1</sup>

<sup>1</sup>Bavarian Health and Food Safety Authority, National Reference Centre for *Borrelia*, Oberschleißheim, Germany

The genus *Borrelia* was first described by Swellengrebe 1907 (Skerman et al., 1989) with *Borrelia anserina* as type species. The genus currently comprises 42 named species (LPSN bacterio.net) including 21 species within the relapsing fever-associated group (RF), 20 species within the Lyme borreliosis-associated group (LB), and one species (*B. turcica*) within the reptile-associated group. There is a wide diversity of borreliae not represented by officially named species, some of which form deeply branching unique monophyletic lineages within the genus.

In 2014 it was proposed to separate the genus *Borrelia* based on analyses of conserved signature insertions and deletions (CSI) and conserved signature proteins (CSP) of two of the groups, i.e. RF and LB species. This proposal was met with resistance from people working in the field and concerns for patient safety were raised. It was further challenged by reports using different methods.

## 2. Objectives

To summarize arguments for and against the recent genus separation proposed for *Borrelia* and to highlight the increasing diversity known to occur in the genus *Borrelia*.

## 3. Results

The percentage of conserved proteins (POCP) was used to show that all groups, RF, LB and reptile-associated, belong to the same genus. A second approach used phyloproteomics to conclude independently that a genus separation of *Borrelia* was not supported. There is overlap in ecology, clinical aspects and molecular features between clades that argue against splitting of the genus *Borrelia*. Information on members of the genus *Borrelia* is increasing but detailed knowledge on relevant features is available only for a small subset of species.

## 4. Conclusion

Current evidence and the increasing diversity that is being recognized in *Borrelia* suggest that division of this genus may not be justified at all, but is certainly premature. As consensus on a universal concept for bacterial taxonomy has not been reached, such taxonomic matters cannot be solved by individual research groups. Systematics is a man-made system to serve the community, thus the impact of decisions must be carefully evaluated by an interdisciplinary approach.

### 080-EMV

Microbial biofilm formation and degradation of the UV absorber octocrylene

\*P. Busch<sup>1</sup>, M. Suleiman<sup>1</sup>, C. Schröder<sup>1</sup>, M. Kuhn<sup>2</sup>, A. Simon<sup>3</sup>, A. Stahl<sup>3</sup>, H. Frerichs<sup>3</sup>, G. Antranikian<sup>1</sup>

<sup>1</sup>Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany

<sup>2</sup>Beiersdorf Aktiengesellschaft, Hamburg, Germany

<sup>3</sup>Hamburg University of Technology, Central Laboratory of Analytical Chemistry, Hamburg, Germany

**Introduction:** Synthetic organic filters are widely used as UV absorbers in sunscreens. The accumulation of those filters, like octocrylene (OC), and their ecological consequences have been discussed recently. OC was detected in wastewater, oceans and some animals. To date, nothing, however, is known about the potential of microorganisms to degrade OC.

**Objectives:** In this study, sediment and water samples from a landfill were used for enrichment cultures identifying the potential of microorganisms to degrade the synthetic organic filter OC.

**"Materials and methods":** Environmental samples from a sewage plant and soakage at a landfill site (Singhofen, Germany) were taken and used for enrichment cultures (inoculation 1 % (v/v) with 0.35 % (v/v) OC as carbon source). Microbial diversity of the cultures was investigated by DGGE analysis. The transformation of octocrylene with selected strains was analyzed using GC-MS and LC-MS/MS.

**Results:** Enrichment cultures showed massive biomass formation on OC droplets. Four genera, assigned to *Gordonia*, *Mycobacterium* and *Hydrogenophaga* were identified by DGGE analysis of the OC-colonized cultures. By applying a "Reverse Discovery" approach the most potent strains *M. agri* and *G. cholesterolivorans* were identified. In the presence of *M. agri* a decrease of 19.1 % of OC compared to the control was observed by GC-MS after 10 days of cultivation. Performing LC-MS/MS analysis the transformation products DOCCA, CPAA and 5OH-OC were detected.

**Conclusion:** In this study, it was shown that applying a "Reverse Discovery" approach microorganisms can be identified for the degradation of hardly degradable synthetic chemicals such as OC. The most potent strain was *Mycobacterium agri* (Suleiman M., Schröder C., Kuhn M., Simon A., Stahl A., Frerichs H. and Antranikian G., 2019, Communications Biology, accepted).

---

### 081-EMV

Effect of fertilizer regimes on microbial degradation of glyphosate in agricultural soil

\*S. Aslam<sup>1</sup>, L. Adrian<sup>1</sup>, K. Nowak<sup>2</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Isotope Biogeochemistry, Leipzig, Germany

<sup>2</sup>Technische Universität Berlin, Geobiotechnology, Berlin, Germany

The increasing use of pesticides has led to the contamination of the major environmental compartments including soil, water and the atmosphere. Glyphosate is one of the most widely used herbicide worldwide. Recently, the International Agency for Research on Cancer (IARC) has classified this molecule as probably carcinogenic to humans which indicates its potential toxicity to ecosystem as well. AMPA and sarcosine pathways are the two widely reported pathways of glyphosate biodegradation with different environmental implications. However, the environmental fate of two glyphosate degradation products namely sarcosine and AMPA is widely unknown. This study elucidates the biodegradation patterns of these two degradation products in agricultural soil. In addition, effect of mineral fertilizers (additional N and P source) on glyphosate biodegradation and formation of non-extractable residues was studied. A soil sampled from an agricultural plot not having prior fertilizer application was fertilized with triple superphosphate (P source) and <sup>15</sup>N labeled or unlabeled Ammonium nitrate (P source). Fertilized and unfertilized soils were then spiked with <sup>13</sup>C and/or <sup>15</sup>N labeled molecules of glyphosate, AMPA, sarcosine and glycine at 50 mg kg<sup>-1</sup> soil. Spiked soil was incubated at 20°C in dark for a period of 75 days. During this period, compound mass balance including mineralization (CO<sub>2</sub>), extractable & non-extractable residues and biomarker analyses (Phospholipid fatty acids and Amino acids) were estimated. Results have shown faster mineralization of glycine and sarcosine followed by glyphosate while AMPA had minimum mineralization (less than 9% even after 75

days). More than 95% of glycine and sarcosine mineralization occurred during first 10 days of incubation. Nitrogen fertilization in soil enhanced the glyphosate mineralization. Biomarker analyses were also conducted to see the incorporation of <sup>13</sup>C and <sup>15</sup>N from the compound into microbial biomass. This study concluded that AMPA could be an environmental concern if glyphosate degradation follows AMPA pathway.

---

#### 082-EMV

The author has not agreed to a publication.

---

#### 083-EMV

The author has not agreed to a publication.

---

#### 084-EMV

##### **The Degradation Pathway of 2-Methylpropene (2-MP) 2-MP is elucidated on the genetic level in *M. gordonae* IBE200: A 6-component T4MO/isoprene-like Monooxygenase is the key enzyme initiating the catabolic sequence**

\*S. Helbich<sup>1</sup>, K. H. Engesser<sup>2</sup>, D. Pieper<sup>3</sup>, K. Sohn<sup>4</sup>

<sup>1</sup>ISWA / Uni Stuttgart, Biological Waste Air Purification, Stuttgart, Germany

<sup>2</sup>ISWA, Biological Waste Air Degradation, Stuttgart, Germany

<sup>3</sup>Helmholtz Centre for Infection Research, AG Mikrobielle Interaktionen und Prozesse., Braunschweig, Germany

<sup>4</sup>Fraunhofer-Institut für Grenzflächen- und Bioverfahrenstechnik IGB, Functional Genomics, Stuttgart, Germany

**1. Introduction** Methylpropene (2-MP) is used as precursor for polymer synthesis, MTBE/ETBE production, or as propellant. It is produced by catalytic dehydrogenation of isobutane or in genetically engineered *E. coli* by fermentation from sugars. From activated sludge of an industrial WWTP we isolated a 2-MP degrading *M. gordonae* IBE200. Kottgoda et al. (2015) (doi:10.1128/AEM.03103-14) also reported a 2-MP degrading *Mycobacterium* sp. ELW1, the respective pathway proposal however based solely on metabolites excreted.

**2. Question** The degradation pathway of 2-MP was to be elucidated on the genetic level in *M. gordonae* IBE200 as there are no metabolites excreted.

**3. Methods** Substrate screening, multiple sequence alignment (MSA), inverse PCR (iPCR)/genome walking, whole genome sequencing, transcriptome analysis, gene disruption/knock-out, heterologous expression.

**4. Results** Grows on: 2-MP ( $\mu=0,03$  h<sup>-1</sup>), n-alkanes (C6-C17), n-alcohols (C3-C6), but not with sugars, rich media, postulated metabolites, aromatics, isoprenoids and terpenes. In cometabolism with 2-MP, n-butane is oxidized subterminally!

MSA with oligos for alkene MOs and PCR resulted in a 1.5 kbp MMO  $\alpha$ -subunit. Genome walking and iPCR resulted in a 10 kbp area containing a full 4-subunit sMMO including a two-component system response regulator. Full genome sequencing at HZI<sup>3</sup>, Braunschweig, revealed a 6-component T4MO/isoprene-like MO next to the sMMO, epoxide hydrolase, alcohol- and aldehyde dehydrogenases, CoA-ligase, 3-component CoA-mutase (ICM) and a complete cluster for cobalamin synthesis. A degradation pathway was postulated, matching the metabolite based findings in ELW1, yet leaving unclear which MO was used. Transcriptome upregulation analysis (1-hexanol and 2-MP grown cells) at FHG<sup>4</sup> Stuttgart verified the postulated pathway, favoring T4MO/isoprene-like MO. Gene disruption/knock-out: T4moA and lcmA with pK19mobsacB/pST-KO: didn't work yet. Heterologous Expression: T4MO and ICM with VLB120 and

other hosts and plasmids revealed isobutyleneoxide formation only in *Pseudomonas veronii* MEK700 pCom10-t4mo.

**5. Conclusions** This is the first 6-component T4MO/isoprene-like MO expressed in an active state.

---

#### 085-EMV

Evidence of a novel side-chain degradation pathway during bile-salt degradation in *Novosphingobium* sp. strain Chol11

\*F. M. Feller<sup>1</sup>, L. Wöhlbrand<sup>2</sup>, R. Rabus<sup>2</sup>, B. Philipp<sup>1</sup>

<sup>1</sup>Westfälische Wilhelms-Universität Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany

<sup>2</sup>Carl von Ossietzky Universität Oldenburg, Institut für Chemie und Biologie des Meeres, Oldenburg, Germany

Bile salts are steroids in the digestive tract of vertebrates and have a C5 carboxylic side chain. In the environment, they are subject to bacterial degradation. Aerobic bile salt degradation may proceed via different pathways: via  $\Delta^{1,4}$ -3-keto intermediates and 9,10-*seco* cleavage used by *Pseudomonas stutzeri* Chol1 or via  $\Delta^{4,6}$ -3-keto-intermediates used by *Novosphingobium* sp. strain Chol11. Side chain degradation is crucial for complete degradation in both pathways. In Gram-negative bacteria using the  $\Delta^{1,4}$ -pathway, the side chain is degraded by two sets of enzymes for aldolytic cleavage, with the first set releasing acetyl-CoA and the second set releasing the remaining side chain as propionyl-CoA from the steroid skeleton. However, neither genes nor intermediates of these reactions are found in strain Chol11. Therefore, proteome analysis of strain Chol11 was performed using the bile salt cholate and the intermediate 7 $\alpha$ ,12 $\beta$ -dihydroxy-androsta-1,4-diene-3,17-dione (DHADD) without side chain as substrates. With this analysis, a distinct up-regulated putative side chain degradation cluster was found containing genes for a putative acyl-CoA dehydrogenase (ACAD, *nov2c221/222*) and an enoyl-CoA hydratase (*nov2c219/220*) as well as the already known steroid CoA ligase *Scf1* (*nov2c230*). In cross-complementation experiments with mutants of *P. stutzeri* Chol1, it was possible to re-establish complete cholate degradation by expressing *nov2c221/222* in the respective ACAD deletion mutant *P. stutzeri* Chol1  $\Delta scd1A$ . A Chol11 deletion mutant of one ACAD component ( $\Delta nov2221$ ) was not able to grow with cholate, but with DHADD without side chain. During growth of mutant strain Chol11  $\Delta nov2c221$  with cholate, an unknown intermediate accumulated, which is currently being identified. These results support a side chain dehydrogenating function of the ACAD. However, the lack of a second ACAD and of aldolytic enzymes known from *P. stutzeri* Chol1 points at a different mechanism for side chain degradation which could be wide-spread in steroid-degrading proteobacteria. Therefore, further genes in strain Chol11 are currently being analysed for their role in side chain degradation.

---

#### 086-EMV

##### ***Pseudomonas* spp. are key players in agricultural biogas substrate degradation**

C. Buettner<sup>1</sup>, M. von Bergen<sup>2</sup>, N. Jehmlich<sup>2</sup>, \*M. Noll<sup>1</sup>

<sup>1</sup>Coburg University of Applied Sciences, Institute for Bioanalysis, Coburg, Germany

<sup>2</sup>Helmholtz-Centre for Environmental Research – UFZ GmbH, Department of Molecular Systems Biology, Leipzig, Germany

Anaerobic degradation (AD) of heterogeneous agricultural substrates is a complex process involving a diverse microbial community. While microbial community composition of a variety of biogas plants (BPs) is well described, little is known about metabolic processes and microbial interaction

patterns. Here, we analyzed 16 large-scale BPs using metaproteomics. All metabolic steps of AD were observed in the metaproteome, and multivariate analyses indicated that they were shaped by temperature, pH, volatile fatty acid content and substrate types. Biogas plants could be subdivided into hydrogenotrophic, acetoclastic or a mixture of both methanogenic pathways based on their process parameters, taxonomic and functional metaproteome. Network analyses showed large differences in metabolic and microbial interaction patterns. Both, number of interactions and interaction partners were highly dependent on the prevalent methanogenic pathway for most species. Nevertheless, we observed a highly conserved metabolism of different abundant *Pseudomonas* spp. for all BPs indicating a key role during AD in carbohydrate hydrolysis irrespectively of variabilities in substrate input and process parameters. Thus, *Pseudomonas* spp. are of high importance for robust and versatile AD food webs, which highlight a large variety of downstream metabolic processes for their respective methanogenic pathways.

## 087-EMV

### Why should we care about microorganisms in the bentonite back fill material for the storage of high-level radioactive waste in deep geological repositories?

\*N. Matschiavelli<sup>1</sup>, M. Dressler<sup>1</sup>, T. Neubert<sup>1</sup>, S. Kluge<sup>1</sup>, A. Schierz<sup>1</sup>, A. Cherkouk<sup>1</sup>

<sup>1</sup>Helmholtz-Zentrum Dresden-Rossendorf, Biogeochemistry, Dresden, Germany

Every year 12,000 metric tonnes of high-level radioactive waste (HLW) are produced worldwide. For the long-term storage of this highly radiotoxic waste, a deep geological disposal by using multiple barriers is favored. Bentonite is proposed as a potential material for sealing the space between the canister containing the HLW and the surrounding host rock. In order to investigate the microbial diversity and metabolic activity of naturally occurring microorganisms as well as their time-dependent evolution, we conducted anaerobic microcosm experiments containing bentonite and a synthetic Opalinus Clay pore water solution. During the one-year incubation at 30 and 60 °C, lactate- or H<sub>2</sub>-stimulated microcosms at 30 °C showed the dominance and activity of strictly anaerobic, sulfate-reducing and spore-forming microorganisms. The subsequent generation of hydrogen sulfide gas in the respective set ups, led to the formation of fractures and iron-sulfur precipitations. In microcosms that incubated at 60 °C, thermophilic bacteria dominated, independent from the availability of substrates. In the respective microcosms, no significant metabolic activity was detected and there was no change in the analyzed biogeochemical parameters. Our results show that indigenous microorganisms evolve in a temperature- and substrate-dependent manner. Potentially formed metabolites could affect the dissolution behavior of minerals and ions within the bentonite as well as the corrosion process of the canister material and require further investigations.

## 088-HYV

### Colonization after MRSA-contacts – Is prophylactic separation of contact patients reasonable?

\*A. Kühn<sup>1</sup>, P. Schipper<sup>1</sup>, B. Schock<sup>1</sup>, J. Stahlmann<sup>1</sup>, I. F. Chaberny<sup>1</sup>

<sup>1</sup>Universitätsklinikum Leipzig, Institut für Hygiene, Krankenhaushygiene und Umweltmedizin, Leipzig, Germany

**Introduction:** MRSA induced infections are associated with increased mortality. Accordingly, risk adapted MRSA screening is performed in in-patient areas at the University Hospital Leipzig (UKL) as recommended by the Commission

for Hospital Hygiene and Infection Prevention (KRINKO) at the Robert Koch Institute. When identifying a carrier of MRSA several infection prevention measures are implemented, including separation of contact patients until negatively tested on MRSA. This procedure established more than 15 years ago at UKL is expensive and means efforts for the staff in treatment of these patients. There is no recommendation of the KRINKO regarding dealing with contact patients.

**Objectives:** How often occurs a pathogen transmission from patients with MRSA colonization to their roommates? Due to this hypothesis is a prophylactic separation of contacts appropriate?

**Methods:** To quantify the rate of MRSA transmission from indices to their contact patients, we collected all patients with nasopharyngeal MRSA colonization as well as their roommates from 2016 to 2018. Contact patients were observed over a six-month period after contact to a MRSA colonized patient. MRSA isolates of index and contact patients were *spa*-typed.

**Results:** In total 227 MRSA contact patients were included. At least 86,8% (n=197) were monitored once in a two-week period after contact. Another 4,8% (n=11) were tested on MRSA in a period of six months after contact. Contact patients were provided with one up to 15 control swabs. Altogether three contact patients were identified with MRSA. *Spa*-typing of two isolates showed different *spa*-types of the contact's isolates compared to their related index. In the third case, there was one positive PCR. Without a cultural isolate the *spa*-type was non-determinable.

**Conclusion:** Considering constantly high number of nasal swabs and declining overall prevalence of MRSA at the UKL, with a tracing rate of 91,6% no transmissions from indices on contact patients were detected. Our study revealed that preemptive separation of MRSA contacts is unnecessary. Therefore resources and costs can be saved.

## 089-HYV

### Results of the prevalence study on the occurrence of multi-drug resistant organisms in Saxony 2017/2018

\*L. Sommer<sup>1</sup>, T. Hackel<sup>2</sup>, A. Hofmann<sup>1</sup>, J. Hoffmann<sup>2</sup>, E. Hennebach<sup>3</sup>, B. Köpke<sup>4</sup>, W. Sydow<sup>4</sup>, I. Ehrhard<sup>2</sup>, I. F. Chaberny<sup>5</sup>

<sup>1</sup>Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, Humanmedizin, Chemnitz, Germany

<sup>2</sup>Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, Humanmedizin, Dresden, Germany

<sup>3</sup>Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, IT, Dresden, Germany

<sup>4</sup>Sächsisches Staatsministerium für Soziales und Verbraucherschutz, Referat 23, Dresden, Germany

<sup>5</sup>Universitätsklinikum Leipzig, Institut für Hygiene, Krankenhaushygiene und Umweltmedizin, Leipzig, Germany

**Introduction:** Multi-drug resistant organisms (MDRO) play an important role in the German healthcare system. Their occurrence is partly registered via the obligation to report, but data on the colonisation of the population are largely lacking.

**Objectives:** The prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA), multi-resistant gram-negative bacteria (MRGN) and vancomycin-resistant enterococci (VRE) was to be determined in three study groups (hospital patients, residents in nursing homes for the elderly and patients in medical practices). In addition, risk factors for carriage of MDRO should be investigated.

**Materials & Methods:** Screening for MDRO was performed as a point prevalence study in voluntarily participating persons via nasal, pharyngeal and rectal swabs or stool samples in 25 hospitals, 14 nursing homes for the elderly as well as 33 medical practices in 12 of 13 districts of Saxony. Suspicious isolates were further examined phenotypically and molecularly. Each participant completed a questionnaire on possible risk factors for MDRO colonization. The data were statistically evaluated by correlation analyses.

**Results:** 1.718 persons, 629 from hospitals, 498 from nursing homes and 591 from medical practices, were examined. In total MDRO was detected in 8.4 % of all participants. 1.3 % persons were tested positive for MRSA, 5.2 % for 3MRGN, 0.1 % for 4MRGN and 2.3 % for VRE. Nine persons were colonized with more than one MDRO. The following independent risk factors could be significantly associated with the detection of MDRO: presence of a degree of care, male sex, current antibiosis, antibiosis within the last 6 months, current tumor disease, peripheral artery disease as well as urinary incontinence. There was no significant correlation between MDRO detection and hygiene characteristics in clinics and nursing homes.

**Conclusion:** To our knowledge, this study represents the first survey on prevalence of different multiresistant pathogen groups in three study groups including outpatients in Germany. 3MRGN were most frequently detected and were also found in younger age groups. VRE were found almost exclusively in the surrounding of individual clinics.

## 090-HYV

Genome-based surveillance of clinical vancomycin-resistant *Enterococcus faecium* (VRE) reveals increased prevalence of vanB-type isolates of ST117/CT71 in German hospitals, 2010-2016

\*B. Neumann<sup>1</sup>, M. Kresken<sup>2,3</sup>, G. Werner<sup>1</sup>, +R. Working Party<sup>4</sup>

<sup>1</sup>Robert Koch Institute, Department of Infectious Diseases, Division of Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany

<sup>2</sup>University of Applied Sciences Cologne, Cologne, Germany

<sup>3</sup>Antinfectives Intelligence GmbH, Rheinbach, Germany

<sup>4</sup>Paul-Ehrlich-Society for Chemotherapy, Rheinbach, Germany

**Background:** Enterococci are considered as common cause of nosocomial infections, where treatment options are limited due to intrinsic and acquired resistances. Particularly vancomycin-resistant *E. faecium* (VRE) represent a burden, since well-adapted lineages are widespread established in healthcare facilities. In recent years, the upcoming resistance to linezolid diminishes this last-line treatment option.

**Objectives:** Microbiological and genome-based analysis of VRE clinical isolates collected as part of the tri-annual resistance studies of the Paul-Ehrlich-Society performed at 25 medical laboratories in Germany, Austria and Switzerland in 2010, 2013 and 2016.

**Materials and methods:** A total of 166 VRE isolates was collected. Susceptibility to 18 antibiotics was tested by applying the broth microdilution method. Resistance breakpoints were assessed according to EUCAST guidelines (v. 9.0). Whole genome sequencing was realized with Illumina technology. For high-resolution genotyping and to determine phylogenetic relations, cgMLST analyses were performed with the generated data.

**Results:** Different genotypes were identified and were assigned to distinct clusters in a phylogenetic tree, showing individual population composition for the different years.

From 2010 to 2016, a change in vancomycin-resistance type from *vanA* to *vanB* was observed. In 2016, the predominant lineage was genotype ST117/CT71, showing the *vanB*-type; the predominant genotype detected in 2010 (ST192, *vanA*) was almost absent in 2016, while ST203 with *vanA*-type was present in all study years.

**Conclusion:** Structured surveys like the PEG resistance studies allow a snapshot of hospital pathogens' prevalence within a given time frame and geographical coverage. We observed the fluctuation of genetic lineages over time, like the rise of ST117 showing the *vanB*-type. cgMLST analyses determined various subtypes within ST117 such as CT71, which in turn ascertained a cross-hospital, regional or country-wide spread of distinct VRE strain types. At the same time, some lineages were present in all cohorts.

## 091-HYV

The ongoing challenge of vancomycin-resistant

*Enterococcus faecium* in Germany and Europe: an epidemiological analysis of blood stream infections

\*O. Ayobami<sup>1</sup>, N. Willrich<sup>1</sup>, I. Noll<sup>1</sup>, A. Reuss<sup>1</sup>, T. Eckmanns<sup>1</sup>, R. Markwart<sup>1</sup>

<sup>1</sup>Robert Koch-Institut, Fachgebiet 37: Nosokomiale Infektionen, Surveillance von Antibiotikaresistenz und -verbrauch, Berlin, Germany

**Introduction:** Vancomycin-resistant *Enterococcus faecium* (VREF) is of great public health significance due to limited therapeutic options. Recently, rising proportions of vancomycin resistance in enterococcal infections have been reported worldwide.

**Objectives:** We investigated time trends of VREF in Germany and Europe and sought to identify factors associated with an increased likelihood of vancomycin resistance.

**Material and Methods:** Data from routine vancomycin susceptibility testing of clinical blood culture *E. faecium* isolates from Germany (n=10,468) and all remaining 29 EU/EEA countries (n=51,071) in 2012-2018 were analysed using descriptive statistics and multivariable regression analyses. Data were obtained from the *European Antimicrobial Resistance Surveillance Network*. VREF proportions were expressed as the proportion of *E. faecium* isolates resistant to vancomycin among all selected isolates.

**Results:** In Germany the proportion of VREF continuously increased from 9.1% (95%CI 6.9-12.0%) in 2014 to 24.4% (95%CI 21.0-28.1%) in 2018. The steady rise was also seen across the remaining EU/EAA countries, where VREF proportions increased from 6.8% (95%CI 5.4-8.5%) in 2012 to 18.5% (95%CI 15.7-21.7%) in 2018. Although rising trends of VREF were observed in all European regions, marked regional differences were found. For 2018, Western Europe showed the lowest VREF proportions (11.3% [95%CI 8.7-14.4%]), while Eastern Europe recorded the highest VREF proportions (33.0% [95% CI 28.0-38.5%]). Multivariable regression analysis showed that adults (>20yrs) had an increased likelihood of VREF compared to children and adolescents (1-20yrs). Hospital units, such as ICUs (OR: 2.6 [95%CI 1.3-5.1]) and internal medicine units (OR: 2.0 [95%CI 1.0-3.7], p = 0.039) were associated with an increased likelihood of VREF compared to the emergency department, which reflects patients with community origin of *E. faecium* infections.

**Conclusion:** VREF proportions are increasing in Germany and across all European regions. Increased awareness and

efforts directed at infection control and antibiotic stewardship are needed, particularly in hospitals, to combat the spread of VREF in Europe.

## 092-HYV

Detecting associations of antibiotic consumption and resistance in infections with *E.coli* with ARVIA – a new surveillance tool for integrated analysis of data from ARS and AVS

\*A. Hoffmann<sup>1</sup>, M. J. Schneider<sup>1</sup>, M. Feig<sup>1</sup>, B. Zacher<sup>1</sup>, H. P. Blank<sup>1</sup>, I. Noll<sup>1</sup>, B. Schweickert<sup>1</sup>, K. Gröschner<sup>1</sup>, M. Abu Sin<sup>1</sup>, T. Eckmanns<sup>1</sup>

<sup>1</sup>Robert-Koch Institut, Surveillance of antibiotic resistance and consumption, Berlin, Germany

**Introduction** ARVIA ("ARS and AVS – integrated analysis") is a new surveillance tool offering an integrated analysis of antibiotic consumption and resistance in hospitals based on data from the German antibiotic consumption surveillance (AVS) and antibiotic resistance surveillance (ARS) to support antibiotic stewardship. Analysis is provided for *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, coagulase-negative *Staphylococci*, *Streptococcus pneumoniae*, *Enterococcus faecium*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex. Hospitals can access their results through an password-protected interactive database on the ARS-website.

**Objective** To evaluate ARVIA for detecting associations between consumption of antibiotics recommended for treatment of infections with *E.coli* and according resistances in participating hospitals.

**Methods** Data are analysed by time series analysis applying Poisson and logistic regression models as appropriate, with significance set at  $p \leq 0.01$ . Furthermore a test for trend is applied to describe evidence if there is a trend in antibiotic resistance not explained by antibiotic consumption.

We analyzed data from the first eleven hospitals participating in ARVIA. We describe the frequency of positive associations between consumption of First-, Second-, Third-, Fourth-generation cephalosporins, fluoroquinolones, carbapenems, aminoglycosides, tigecycline and sulfonamide/trimethoprim for treatment of infections with *E.coli* and according resistance, with no evidence for a variable other than antibiotic consumption affecting changes in resistance in the test for trend.

**Results** Positive associations were found for *E.coli* and the consumption of the following antibiotic groups (n=number of hospitals): Second-generation cephalosporins (n=2), Third-generation cephalosporins (n=3), aminoglycosides (n=3), fluoroquinolones (n=3), penicillins (n=5) and sulfonamide/trimethoprim (n=3).

**Conclusion** ARVIA is able to detect associations between consumption of clinical relevant antibiotics and according resistance in *E.coli* and is therefore a tool that can support antibiotic stewardship in hospitals.

## 093-HYV

Ongoing supraregional outbreak of clonal OXA-244-carbapenemase-producing *Escherichia coli* (CPE) in Germany, 2017-2019

\*K. Kremer<sup>1</sup>, R. Kramer<sup>2</sup>, J. B. Hans<sup>3</sup>, S. Haller<sup>2</sup>, N. Pfennigwerth<sup>3</sup>, Y. Pfeiffer<sup>2</sup>, B. Neumann<sup>2</sup>, G. Werner<sup>2</sup>, T. Eckmanns<sup>2</sup>, S. Gatermann<sup>3</sup>

<sup>1</sup>Robert Koch Institute, Postgraduate Training for Applied Epidemiology (PAE), Berlin, Germany

<sup>2</sup>Robert Koch Institute, Berlin, Germany

<sup>3</sup>National Reference Centre (NRC) for multidrug-resistant gram-negative bacteria, Medical Microbiology, Bochum, Germany

## Background:

Infections with carbapenemase-producing Enterobacterales (CPE) lead to considerable increased mortality and morbidity. Outbreaks occur mainly in hospitals and are most often due to person-to-person transmission. From 2017 to 2019 an increase of *Escherichia coli* with carbapenemase OXA-244 was detected at the German National Reference Centre for multidrug-resistant gram-negative Bacteria (NRC). We conducted an outbreak investigation to analyse spread and route of transmission.

## Methods:

CPE infections and/or colonisations are notifiable and diagnostic laboratories are requested to send CPE-isolates to the NRC. We defined cases as patients with confirmed detection of OXA-244-producing *E.coli* from 2017 to 2019 in Germany. Whole genomes of OXA-244-*E.coli*-isolates were sequenced (Illumina) and analysed by multilocus-sequence-typing (MLST), core genome (cg) MLST and high-resolution SNP analyses. For epidemiological analyses RStudio and RegioGraph-software were applied. We collected case information on hospital stays, diagnosis and recent stays in other countries through a standard questionnaire for hospitals and cases.

## Results:

The NRC detected 32 OXA-244-*E.coli*-isolates in 2017; 91 in 2018 and 110 until August 2019. cgMLST and SNP analyses of 152 selected isolates from 2017 to 2019 revealed 20 clusters of closely related strains. One major cluster harboured 63 ST38-*E.coli* isolates; 39 correspond to data in the national notification system. Among these 39 cases 11 had infections, 6 of them bloodstream infections. Further male and female of all age-groups were equally represented, living and treated in 10 federal states of Germany. First 19 completed questionnaires showed that 71% of the patients were previously hospitalised and 69% had their family or a recent stay abroad.

## Conclusions:

We confirmed occurrence of closely related ST38-*E.coli* isolates with OXA-244 carbapenemase over a period of three years in German hospitals. Lack of an epidemiological link and the fact that 29% of the cases had not been hospitalised previously might indicate transmission other than by continuous spread from person to person or a non-healthcare-associated CPE-outbreak.

## 094-MBTV

Optimized bioprocess for production of high-value omega-3 fatty acids using recombinant *Yarrowia lipolytica*

\*D. Dietrich<sup>1</sup>, S. Jovanovic<sup>1</sup>, K. Gempeler<sup>2,3</sup>, M. Kohlstedt<sup>1</sup>, S. Wenzel<sup>2,3</sup>, R. Müller<sup>2,3</sup>, C. Wittmann<sup>1</sup>

<sup>1</sup>Saarland University, Institute of Systems Biotechnology, Saarbrücken, Germany

<sup>2</sup>Saarland University, Institute of Pharmaceutical Biotechnology, Saarbrücken, Germany

<sup>3</sup>Helmholtz Institute for Pharmaceutical Research Saarland, Saarbrücken, Germany



## 1 Introduction

Long-chain polyunsaturated fatty acids (LC-PUFAs) have a variety of health benefits and enable various applications in the food and pharmaceutical industry. Docosahexaenoic acid (DHA) is a LC-PUFA of recognized value. The omega-3 fatty acid is a primary structural component of the human brain, cerebral cortex, skin and retina, thus displaying a high-value food supplement [1]. A promising approach to derive LC-PUFAs is fermentative production using the oleaginous yeast *Yarrowia lipolytica*. The microbe naturally accumulates fatty acids and has proven good accessibility to genetic modification [2].

## 2 Objectives

Bioengineering has recently enabled recombinant *Y. lipolytica* strains, which express myxobacterial PUFA synthase cluster and accumulate LC-PUFAs [3]. Here, we focused on the development of a superior bioprocess to derive DHA using recombinant *Y. lipolytica*.

## 3 Materials & methods

An interdisciplinary approach of systems metabolic engineering was used.

## 4 Results

The production of DHA was successfully demonstrated, using different strains of *Y. lipolytica*, which expressed synthetic PUFA gene clusters. The created strains accumulated up to 104.8 mg/L in shake flasks. Subsequent bioprocess and medium design resulted in a fed-batch fermentation process, which yielded a DHA titer of 350 mg/L. Hereby, the synthesis of DHA was strongly enhanced among all fatty acids, which enabled a DHA content of 16.8 % of the total fatty acid pool [4].

## 5 Conclusion

Efficient production of DHA was achieved. Given further rounds of optimization, the developed approach has promising potential to enable industrial production of this high-value omega-3 fatty.

--

[1] Abedi, E., & Sahari, M. A. (2014). Food science & nutrition, 2(5), 443-463. doi: 10.1002/fsn3.121

[2] Gemperlein, K. (2014). (Dissertation), Saarland University. doi:10.22028/D291-23027

[3] Nicaud, J. M. (2012). Yeast, 29(10), 409-418. doi: 10.1002/yea.2921

[4] Gemperlein, K., Dietrich, D., Kohlstedt, M., Zipf, G., Bernauer, H. S., Wittmann, C., Wenzel, Silke C., Müller, R. (2019). Nature Communications, 10(1), 4055. doi: 10.1038/s41467-019-12025-8

### 095-MBTV

A new metallophore from *Variovorax paradoxus* EPS

\*M. Hofmann<sup>1</sup>, L. Malik<sup>1</sup>, J. H. Dietrich<sup>2</sup>, M. Nett<sup>2</sup>, D. Tischler<sup>1,3</sup>, T. Heine<sup>1</sup>

<sup>1</sup>TU Bergakademie Freiberg, Ecological Center, Freiberg, Germany

<sup>2</sup>TU Dortmund University, Department of Biochemical and Chemical Engineering, Dortmund, Germany

<sup>3</sup>Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany

Metallophores are small metal chelating compounds produced by many microorganisms under iron limiting conditions. Due to their high potential for biotechnological, medical, and environmental applications, there is a great interest to identify and characterize new metallophores. Recently the siderophore production of *Variovorax* spp. was investigated and four different metallophores were found: variobactin, vacidobactin,<sup>1</sup> variochelin,<sup>2</sup> and imaquobactin.<sup>3</sup> In order to further explore the metallophore production of the genus *Variovorax*, the metallophore of *Variovorax paradoxus* EPS was isolated and characterized. Therefore, the metallophore production was tested in different media and with different carbon sources. The metallophore activity was determined using a chrome azurol S assay. The highest amount of metallophore was obtained using low-phosphate minimal medium with glucose as carbon source. The produced metallophore was extracted with XAD and further purified using C<sub>18</sub> columns. Experiments with different light conditions showed a photosensitivity of the compound. Bioinformatic analysis indicated some similarities, but also clear distinctions to the four known metallophores of the genus. This correlates well with the differences in the metallophore gene cluster, which shows a different gene organization and additional NRPS domains compared to metallophore gene clusters of other *Variovorax* strains. Although the exact structure needs to be verified by NMR analysis it is very likely, that we found a structurally new metallophore in *V. paradoxus* EPS.

1 C. W. Johnston, M. A. Skinnider, M. A. Wyatt, X. Li, M. R. M. Ranieri, L. Yang, D. L. Zechel, B. Ma and N. A. Magarvey (2015) Nature Communications, 1–11

2 C. Kurth, S. Schieferdecker, K. Athanasopoulou, I. Seccareccia and M. Nett, Journal of Natural Products (2016) 79, 865–872.

3 A. W. Robertson, N. G. McCarville, L. W. MacIntyre, H. Correa, B. Haltli, D. H. Marchbank and R. G. Kerr, Journal of Natural Products (2018) 81, 858–865.

### 096-MBTV

Utilization of cyanobacterial TCA cycles for the production of *trans*-4-hydroxy-L-proline

\*F. Brandenburg<sup>1</sup>, E. Theodosiou<sup>2</sup>, C. Köster<sup>2</sup>, S. Klähn<sup>2</sup>, A. Schmid<sup>2</sup>, J. Krömer<sup>2</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

<sup>2</sup>Helmholtz Centre for Environmental Research, Leipzig, Germany

In our project, we investigate the capacities of cyanobacterial tricarboxylic acid (TCA) cycles for the sustainable production of *trans*-4-hydroxy-L-proline (Hyp), a valuable chiral building block for the pharmaceutical and cosmetic industry.

The currently used method for production of Hyp is the acidic hydrolysis of mammalian collagen. It requires large volumes of solvent and produces high amounts of waste. Alternative production of Hyp via genetically engineered *E. coli* has been achieved via heterologous expression of  $\alpha$ -ketoglutarate-dependent proline-4-hydroxylases (P4H). Both substrates,  $\alpha$ -ketoglutarate and proline, are part or derivatives of the TCA cycle, respectively, and hence are provided by the host cells metabolism. Similarly, the co-product succinate is part of the

TCA cycle and therefore further metabolized in the cell. However, carbon and energy are provided by the breakdown of glucose.

In autotrophic cyanobacteria, TCA cycles do not serve the same dominant role in provision of chemical energy in the form of ATP and reduction equivalents and thus the cells maintain only low fluxes. In fact, cyanobacterial TCA cycles were considered incomplete as the conversion from  $\alpha$ -ketoglutarate to succinyl-CoA is not taking place. Fairly recently, several bypasses were identified and characterized like the so called cyanobacterial complete TCA cycle shunt via succinic-semialdehyde.

To produce Hyp in *Synechocystis*, we added an additional bypass to the TCA cycle. We fused the gene coding for the P4H of *Dactylosporangium* sp. strain RH1 with the copper-inducible *petE*-promoter, native to *Synechocystis*. In our latest results, we are able to produce significant amounts of Hyp from CO<sub>2</sub> and show that it is possible to utilize the TCA cycle for the production of valuable chemical compounds. In future work we want to use our production strains to better understand the connection and regulation of photosynthesis with the central carbon metabolism, in order to improve the bio-technological applications of cyanobacteria.

## 097-MBTV

### Evaluation of biological phosphorous removal effects in a full scale wastewater treatment plant

\*S. Günther<sup>1</sup>, C. Süring<sup>1</sup>, S. Müller<sup>1</sup>

<sup>1</sup>Helmholtz-Centre for Environmental Research, Environmental Microbiology, Leipzig, Germany

## Question

In the light of diminishing phosphorous (P) resources and mandatory P recovery there is, again, an increasing interest in P elimination strategies of wastewater treatment plants (WWTPs). The choice of P removal strategy will have a big impact upon the choices for recovery processes. Biological P removal may prove beneficial for some of the recovery strategies, since P can be relocated and easily recovered within the WWTP, allowing for cost effective disposal of the process wastes. Here, by means of extensive sampling and bioinformatics, we will highlight the relationship between choice of P elimination strategy and microbial community function in a full scale WWTP

## Methods

A full scale WWTP was sampled at several treatment steps over a long time period. Bulk abiotic parameters were measured. Together with cytometric information of the respective microbial communities bioinformatics analysis give insights on the influence of P concentrations on the microbial community.

## Results

It was found that the effective biological P removal in the WWTP could, under certain circumstances, impair microbiological functions on other parts of the WWTP. While there was a high removal of P in the activated sludge system, the release of P in the anaerobic digester with average *ortho*-P concentrations of 0.6 kg P/m<sup>3</sup> proved critical in a situation where the community was put under an additional stress. Exceedingly high P concentrations led to a complete breakdown of the biogas production process and a very slow recovery of biological functions.

## Conclusion

Biological P elimination in a WWTP has several advantages like the lack of need for precipitation chemicals and precipitation technology and the facilitation of P recovery. On the other hand, high P concentrations, especially in anaerobic digestion systems of such WWTPs, can prove critical for the function of the microbial community. Therefore, either close monitoring of P or a versatile P recovery process is needed. The latter will also help to serve a circular economy by sustainable resource management.

## 098-MBTV

### Bacterial magnetosomes as versatile tool for biomedical and biotechnological applications

\*F. Mickoleit<sup>1</sup>, D. Schüler<sup>1</sup>, V. Jérôme<sup>2</sup>, R. Freitag<sup>2</sup>, C. Jörke<sup>3</sup>, J. H. Clement<sup>3</sup>, S. Geimer<sup>4</sup>

<sup>1</sup>Universität Bayreuth, Lehrstuhl Mikrobiologie, Bayreuth, Germany

<sup>2</sup>Universität Bayreuth, Bioprozesstechnik, Bayreuth, Germany

<sup>3</sup>Universitätsklinikum Jena, Hämatologie und Internistische Onkologie, Jena, Germany

<sup>4</sup>Universität Bayreuth, Elektronenmikroskopie, Bayreuth, Germany

The biosynthesis of magnetosomes by magnetotactic bacteria like the alphaproteobacterium *Magnetospirillum gryphiswaldense* is an intriguing example of a biomineralization process. Magnetosomes are monocrystalline single-domain nanoparticles of chemically pure magnetite enveloped by a proteinaceous phospholipid bilayer (magnetosome membrane) [1]. Because of their unprecedented properties such as high crystallinity, strong magnetization, uniform shape and size and the ability to engineer the magnetosome membrane by genetic means, the particles have been considered for many biomedical and biotechnological applications [2].

For their future use in the biomedical field we investigated potential cytotoxic effects of magnetosomes on eukaryotic cells. The vitality of a FaDu cancer cell line was shown to be not impaired when incubated with wildtype magnetosomes for up to 24 h, and no increased cell death was observed. Furthermore, short-term incubation with magnetosomes was found to be sufficient to allow for magnetic separation, with recovery rates of up to 60%.

In a systematic step-wise bottom-up approach we furthermore developed a versatile genetic toolkit for the generation of functionalized magnetic nanoparticles with several fully genetically encoded properties [3]. The optimized system was subsequently used for magnetosome display of ligands of the TNF receptor superfamily. Biological functionality of the particles could be demonstrated by efficient stimulation of sensor cells expressing the corresponding receptor, suggesting an optimal ligand density to be required for the highest cellular response [4].

Overall, this illustrates the versatile features of (engineered) bacterial magnetosomes, and our results provide a route toward the generation of novel, fully genetically encoded, functionalized biomaterials.

[1] Uebe R., Schüler D. (2016) *Nature Reviews Microbiology*, 14(10), 621.

[2] Mickoleit F., Schüler D. (2018) *Bioinspired, Biomimetic and Nanobiomaterials*, 8(1), 86-98.

[3] Mickoleit F., Schüler D. (2017) *Advanced Biosystems*, 2(1), 1700109.

## 099-MBTV

Multiplexing antibiotic screening in droplet microfluidics using an optofluidic platform

\*S. Hengoju<sup>1,2</sup>, K. Martin<sup>1</sup>, L. Mahler<sup>1</sup>, O. Shvydkiv<sup>1</sup>, M. Tovar<sup>1</sup>, M. Roth<sup>1</sup>, M. Rosenbaum<sup>1,2</sup>

<sup>1</sup>Hans Knoll Institute, Jena, Germany

<sup>2</sup>Friedrich Schiller University, Faculty of Biological Sciences, Jena, Germany

The necessity of novel antibiotics has been in high demand due to the increasing occurrence of multiresistant microbial pathogens. However, ongoing screening efforts for the discovery of novel active compounds have not been successful due to limitations in throughput and operational cost. For empowering antibiotic screening assays from complex microbial communities, we have developed a droplet-based optofluidic platform with integrated optical fibers. We combine our previously developed strategy for culturing microbial strains [1] with multi-color fluorescence detection using optical fibers [2]. Furthermore, a mixture of reporter strains is pico-injected so that millions of picoliter cultures can be screened for inhibition activity based on the independent survival signals of each different reporter. This approach demonstrates multiplexing capability in screening assays aimed at simultaneously discovering new bioactive compounds and exploring their molecular targets.

## References

[1] Mahler et al., 2018, *Sci Rep*.

[2] Tovar et al., 2019, *Anal Chem*.

## 100-MBTV

**Automated Fed-Batch process for microbial lipid production using volatile fatty acids**

X. Qian<sup>1</sup>, M. Jiang<sup>1</sup>, \*K. Ochsenreither<sup>2</sup>

<sup>1</sup>Nanjing Tech University, College of Biotechnology and Pharmaceutical Engineering, Nanjing, China

<sup>2</sup>Karlsruhe Institute of Technology, BLT 2: Technical Biology, Karlsruhe, Germany

### 1. Introduction

Microbial lipids are intracellular storage lipids comprised of triacylglycerols. They have been considered as alternative feedstock for biodiesel and oleochemicals. However, large-scale production of microbial lipids is challenged by high production cost, with feedstock costs as the main factor. Therefore, the exploitation of low-cost substrates for microbial lipids production is needed. Acetate is a waste- or by-product generated from lignocellulosic biomass hydrolysate, syngas and other anaerobic bioprocesses. Although acetate shows promising properties as substrate for biotechnological processes, however, acetate also inhibits microbial growth.

### 2. Objectives

In this study, lipid production from acetate and volatile fatty acids (VFA) as carbon source by using the self-isolated oleaginous yeast *Apiotrichum porosum* DSM 27194 was investigated. Therefore, a novel fermentation strategy using acetate or VFA as the acid reagent to control the pH was

designed and the fatty acid composition under different conditions was analyzed and compared.

### 3. Methods

Automated Fed-Batch fermentations were carried out in 2 L bioreactors. Lipid content and fatty acid composition was analyzed by GC, whereas acid concentration in culture broth was monitored by HPLC

### 4. Results

By supplementing glucose as the stimulant for microbial growth, a lipid content of 35-40 % based on acetate and VFA was obtained, corresponding to a lipid concentration of up to 11.5 g/L. Fatty acid composition of acetate-derived lipids were similar to vegetable oil, suggesting its potential for biodiesel production.

### 5. Conclusion

In this study, *A. porosum* was found to grow well on 10 or 20 g/L of acetate supplemented with 10 g/L of glucose as the stimulant. When acetic acid was used to maintain the pH and replenish the carbon source continuously and slowly, *A. porosum* could accumulate up to 40% lipids per dry biomass. These results demonstrate the promising prospect of economic microbial lipid production from acetate and VFAs through automated fed-batch.

## 101-MBTV

Microbiology impacting coffee culture – Defined fermentation for improving taste and quality of coffee

\*C. Burkhardt<sup>1</sup>, P. Busch<sup>1,2</sup>, C. J. Bolten<sup>3</sup>, G. Antranikian<sup>1</sup>

<sup>1</sup>Technische Universität Hamburg, Institut für Technische Mikrobiologie, Hamburg, Germany

<sup>2</sup>Technical University Hamburg, Institut für Technische Mikrobiologie, Hamburg, Germany

<sup>3</sup>Institute of Material Sciences, Nestlé Research, Department of Biology, Lausanne, Switzerland

### 1. Introduction

Coffee is one of the most popular beverages in Germany and throughout Europe. Quality and taste of coffee is significantly influenced by the postharvest processing (fermentation, drying, roasting). For example, in so-called wet processing coffee cherries are treated unspecifically with naturally occurring microorganisms. Nevertheless, the development of new tastes and flavors and compositional changes of ingredients are limited by these natural procedures. Fermentation of coffee beans with specific microorganisms can provide new opportunities to influence and systematically improve taste of the coffee product.

### 2. Objectives

The aim of the project was to influence taste and quality of coffee by fermentation with defined microorganisms and roasting processes. This included the selection of pure strains and combination of appropriate microorganisms in order to improve the product properties.

### 3. "Materials & methods"

Microbial strains and environmental samples were stepwise adapted to green coffee beans as substrate. Different fermentation conditions (oxic/anoxic, high/moderate

temperatures) were tested. After fermentation, the coffee beans were evaluated by analysis of key ingredients with chromatographic methods.

#### 4. Results

Fermentation conditions had high influence on the composition of coffee bean ingredients. Especially chlorogenic acid was influenced when it was oxidized at elevated temperatures. Several strains were able to growth on coffee and resulted in changed flavor, sugar, organic acid and phenolic acid profiles. Moreover, by enrichment of an environmental sample from hot springs of the Azores and a comparative analysis of the metagenomes, a community shift was observed and key species of coffee fermentation under elevated temperatures were identified.

#### 5. Conclusion

In this proof of concept, we demonstrated the possibility of influencing coffee flavor and ingredient composition with selected microbial strains that have not been used before. Moreover, by a metagenomic approach we identified anaerobic thermophiles which are promising candidates for further fermentation experiments.

#### 102-MPV

Within-host evolution of bovine *Staphylococcus aureus* selects for a SigB-deficient pathotype characterized by reduced virulence but enhanced proteolytic activity and biofilm formation

H. Marbach<sup>1</sup>, K. Mayer<sup>1</sup>, C. Vogl<sup>1</sup>, J. Y. H. Lee<sup>2</sup>, I. Monk<sup>2</sup>, D. O. Sordelli<sup>2,3</sup>, F. R. Buzzola<sup>3</sup>, M. Ehling-Schulz<sup>1</sup>, \*T. Grunert<sup>1</sup>

<sup>1</sup>Veterinärmedizinische Universität Wien, Wien, Austria

<sup>2</sup>University of Melbourne, Melbourne, Australia

<sup>3</sup>Universidad de Buenos Aires and CONICET, Buenos Aires, Argentina

#### 1. Introduction

*Staphylococcus aureus* is a major cause of bovine mastitis, commonly leading to long-lasting, persistent and recurrent infections. Thereby, *S. aureus* constantly refines and permanently adapts to the bovine udder environment. However, the genetic and molecular mechanisms allowing predominant *S. aureus* subtypes to successfully persist even for years inside the bovine udder are far from understood.

#### 2. Objectives

In this study, we investigate *S. aureus* within-host adaptation in bovine persistent mastitis.

#### 3. Materials & methods

We analyzed a set of *S. aureus* isolates, collected over the course of three months from a cow with chronic, subclinical and untreated bovine mastitis. To obtain a comprehensive picture of within-host adaptation, we carried out an in-depth investigation of the evolution of this pathogen within the bovine host including molecular, phenotypic and functional assays.

#### 4. Results

Whole genome sequence analysis revealed a complete replacement of the initial predominant variant by another isogenic variant. We report for the first time within-host

evolution towards a sigma factor SigB-deficient pathotype in *S. aureus* bovine mastitis, associated with a single nucleotide polymorphism in *rsbU* (G368A → G122D), a contributor to SigB-functionality. The emerged SigB-deficient pathotype exhibits a substantial shift to new phenotypic traits comprising strong proteolytic activity and poly-N-acetylglucosamine (PNAG)-based biofilm production. This possibly unlocks new nutritional resources and promotes immune evasion, presumably facilitating extracellular persistence within the host. Moreover, we observed an adaptation towards attenuated virulence using a mouse infection model.

#### 5. Conclusion

This study extends the role of sigma factor SigB in *S. aureus* pathogenesis, so far described to be required for intracellular persistence during chronic infections. Our findings suggest that *S. aureus* SigB-deficiency is an alternative mechanism for persistence and underpin the clinical relevance of staphylococcal SigB-deficient variants which are consistently isolated during human chronic infections.

Reference: Marbach et al. 2019; Sci Rep. 9(1):13479

#### 103-MPV

Inter- and intraclonal diversity in *S. epidermidis* prosthetic joint infectionInter- and intraclonal diversity in *S. epidermidis* prosthetic joint infection

\*A. Both<sup>1</sup>, J. Huang<sup>1</sup>, C. Lausmann<sup>2</sup>, S. Weißelberg<sup>1</sup>, H. Büttner<sup>1</sup>, H. Rohde<sup>1</sup>

<sup>1</sup>University Medical Center Hamburg-Eppendorf, Medical Microbiology, Hamburg, Germany

<sup>2</sup>Helios ENDO Klinik, Hamburg, Germany

Background: While *S. epidermidis* colonizes the skin and nares of virtually all humans, the species also is a prominent pathogen causing foreign material associated infections. A plethora of factors promoting pathogenesis and biofilm formation has been identified. However, extend of and mechanisms behind adaptation processes to an invasive life style have been largely overlooked so far. In the present study, a collection of colonizing and corresponding invasive *S. epidermidis* isolates from PJI cases was used to investigate pathogenicity factors facilitating the establishment of infection, and diversity driven adaptation processes fostering the shift from colonization to invasion.

Methods: Nasal swab samples and intra-operative joint fluid were cultured from all patients included. From each material multiple colonies grown from primary plates (nasal swabs: up to 30; joint fluid: at least three) were picked. Isolates identified as *S. epidermidis* were then analyzed by PFGE and whole genome sequencing. Phylogenetic trees were constructed and genomic association studies were conducted. All isolates were tested for biofilm formation, Agr-activity, cell-cell aggregation, proteolysis, growth characteristics and antibiotic susceptibility.

Results: 133 PJI patients were prospectively included into the study. Twenty-eight cases were caused by *S. epidermidis* and thus selected for in-depth analysis. A total of 267 *S. epidermidis* genomes was analysed (3 to 12 infectious isolates and 1 to 12 commensal isolates per patient). Phylogenies were constructed and a genomic association study conducted. In 6/28 cases a nasal clone identical to the infectious isolate was identified. Interestingly, we observed marked intra-clonal phenotypic heterogeneity within the infection and in significant phenotypic divergence from the nasal clone. Preliminary results point to gene

expression regulation as the basis. Importantly, there was evidence of altered antibiotic susceptibility profiles in subpopulations of *S. epidermidis* within infections.

**Conclusion:** There is pronounced inter- and intra-clonal diversity in PJI caused by *S. epidermidis*.

---

#### 104-MPV

***Staphylococcus aureus*** pathogenicity in cystic fibrosis patients – virulence genes, phylogeny and horizontal gene transfer

J. Lange<sup>1</sup>, D. Görlich<sup>1</sup>, \*B. C. Kahl<sup>2</sup>

<sup>1</sup>University Hospital Muenster, Institute of Biostatistics and Clinical Research, Münster, Germany

<sup>2</sup>University Hospital Münster, Institute of Medical Microbiology, Münster, Germany

**Background:** *S. aureus* is the bacterium with the highest prevalence in the cystic fibrosis (CF) population and it has shown to play a vital role in CF airway inflammation. In light of the clinical significance of *S. aureus* in CF on the one hand and the lack of internationally accepted treatment standards on the other hand, this study aims to identify bacterial factors associated with pulmonary exacerbations.

**Methods:** Respiratory specimens (nose and throat swabs, sputa) from 195 CF patients from 17 centers were studied during an observational prospective multicenter study at a central microbiology laboratory. From every sample, phenotypically different (size, pigmentation, hemolysis) *S. aureus* isolates were subjected to *spa* typing. The presence of virulence genes (*fnbA/B*, *clfA/B*, *cna*, *sdrC/D/F*, *sasG*, *eap*, *emp*, *pvl*, *hlg*, *eta/etb*, *tst-1*, *sea-see*, *seg-sej*) and the 4 *agr* specificity groups were analyzed by single and multiplex PCR. Clinical data were collected and correlated to microbiological data to screen for links between bacterial genetical traits and the patients' clinical outcomes.

**Results:** Neither the number of *S. aureus* clones in the patients' specimens, the presence of prevalent and/or dominant clones nor the quantity of virulence genes of the individual *S. aureus* clone had an impact on clinical parameters. None of the investigated virulence genes was associated with exacerbations. However, for isolates belonging to *agr*-types 1 and 4, there was a link to the clinical status. Moreover, *agr* types showed a clear association with the clonal background of *S. aureus*. Analyses concerning the plasticity of the virulence genes revealed significantly increased potential for horizontal gene transfer in case of high bacterial density and the presence of several and/or related *S. aureus* clones. Furthermore, the data point towards increased rates of horizontal gene transfer in case of antibiotic therapy.

**Conclusions:** Our results provide evidence for the concept that rather the phylogenetic background than the presence of specific virulence gene accounts for differences in *S. aureus* pathogenicity. Horizontal gene transfer was high in patients with environmental stress.

---

#### 105-MPV

***Staphylococcus aureus* pneumonia: preceding influenza infection paves the way for low virulent strains**

\*S. Deinhardt-Emmer<sup>1</sup>, K. F. Haupt<sup>2</sup>, M. Garcia-Moreno<sup>1</sup>, J. Geraci<sup>1</sup>, C. Forstner<sup>3</sup>, M. W. Pletz<sup>3</sup>, C. Ehrhardt<sup>2</sup>, B. Löffler<sup>1</sup>

<sup>1</sup>Jena University Hospital, Institute of Medical Microbiology, Jena, Germany

<sup>2</sup>Section of Experimental Virology, Jena University Hospital,

Germany

<sup>3</sup>Institute for Infectious Diseases and Infection Control, Jena University Hospital, Germany

*Staphylococcus aureus* (*S. aureus*) is a facultative pathogenic bacterium that colonizes the nasopharyngeal area of healthy individuals, but can also induce severe infection, such as pneumonia. Pneumonia caused by mono- or superinfected *S. aureus* leads to high mortality rates. To establish an infection *S. aureus* disposes of a wide variety of virulence factors, which can vary between clinical isolates.

Our study aimed to characterize pneumonia isolates for their virulent capacity. For this, we analyzed *S. aureus* isolates each from nasal colonization, pneumonia due to *S. aureus* as a solely identified pathogen (mono-infection), and pneumonia due to *S. aureus*/influenza virus co-infection. A total of 70 strains were tested for their genes for virulence factors and clonal clusters. Additionally, the host-pathogen interaction was analyzed through several functional assays in cell culture systems of professional and non-professional phagocytes.

Strains from pneumonia due to *S. aureus* mono-infection showed enhanced invasion and higher cytotoxicity against professional phagocytes than colonizing and co-infecting strains. This corresponded to the high presence of cytotoxic components in primary pneumonia strains, such as defined leucocidins, superantigens and serine proteases. By contrast, strains obtained from a coinfection did not exhibit these virulence characteristics and resembled strains from colonization, although they caused the highest mortality rate in patients.

Taken together, our results underline the requirement of adhesins and toxins to cause pneumonia due to *S. aureus* mono-infection, whereas in co-infection even low-virulent strains can lead to pneumonia.

---

#### 106-MPV

Pneumococcal adaptation to the nasopharynx and lung during colonization and infection

M. R. Abdullah<sup>1</sup>, C. Hentschker<sup>2</sup>, D. Dittmar<sup>2</sup>, P. Hildebrandt<sup>2</sup>, S. Michalik<sup>2</sup>, U. Völker<sup>2</sup>, \*S. Hammerschmidt<sup>1</sup>

<sup>1</sup>Universität Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

<sup>2</sup>University Medicine Greifswald, Department of Functional Genomics, Greifswald, Germany

**Introduction:** *Streptococcus pneumoniae* (pneumococci) are versatile pathobionts colonizing the mucosal surface of the upper airways of humans. This is the reservoir for pneumococcal pneumonia, or sepsis and meningitis (PM). Pneumococci adapt perfectly to the various host compartments differing e.g., in nutrients and host defense mechanisms. Thus, the proteome of pneumococci mirrors the conditions in the host compartments. By employing an *in vivo* proteome approach we recently indicated that the competence regulator ComDE and oligopeptide transporter AliB are essential for PM (Schmidt *et al.*, 2019 PLoS Pathog 15:e1007987). Here we deciphered the proteome of pneumococci during colonization and lung infection.

**Methods:** Mice were intranasally infected with GFP-expressing serotype 4 strain TIGR4 or serotype 19F. Pneumococci were recovered from mice by a nasopharyngeal wash or bronchoalveolar lavage (BAL). Pneumococci were separated from eukaryotic cells by FACS sorting onto a filter. After on-filter digestion with trypsin peptides were measured using LC-MS/MS in a data-

independent acquisition mode (DIA) and analysed with the Spectronaut<sup>TM</sup> software package.

**Results:** Three days post-infection we isolated TIGR4 from the nasopharynx and lungs, while we recovered 19F after 3 and 5 from the nasopharynx of infected mice. The *in vivo* proteome analysis of TIGR4 or 19F samples with 1-5 million pneumococci resulted in the identification of approximately 1000 proteins per sample. The *in vivo* proteome profiles were compared to *in vitro* proteome profiles of pneumococci cultured in complex or chemically defined (CD) media. In particular the 19F showed 3 clusters with a clear separation of 19F cultured in CD or complex media and *in vivo* samples. Strikingly, there was no difference between the proteome profiles of 19F colonizing 3 or 5 days the nasopharynx. The strain-dependent differences in protein patterns and niche-specific adaptations will be discussed.

**Conclusion:** Pneumococci adapt to their specific host compartments and protein expression profiles differ from *in vitro* cultures. The differentially produced proteins are identified and their impact on colonization will be analyzed.

### 107-MPV

Identification of drug-resistance determinants in a clinical isolate of *Pseudomonas aeruginosa* by high-density transposon mutagenesis

M. Sonnabend<sup>1</sup>, K. Klein<sup>1</sup>, E. Walter<sup>1,2</sup>, A. Angelov<sup>1</sup>, R. Kluj<sup>2</sup>, C. Mayer<sup>2</sup>, I. Autenrieth<sup>1</sup>, M. Schütz<sup>1</sup>, \*E. Bohn<sup>1</sup>

<sup>1</sup>University of Tübingen, Institut für Medizinische Mikrobiologie und Hygiene, Tübingen, Germany

<sup>2</sup>University of Tübingen, Department of Biology, Microbiology & Biotechnology, Tübingen, Germany

*Pseudomonas aeruginosa* (Pa) is one of the most important nosocomial pathogens. The incidence of multidrug-resistant strains is rising and the treatment options are continuously decreasing because Pa employs various resistance mechanisms including low permeability of the outer membrane and expression of efflux pumps and  $\beta$ -lactamases.

With the aim to identify potential new targets to restore antimicrobial susceptibility of multidrug-resistant (MDR) *Pseudomonas aeruginosa* (Pa), we generated a high-density transposon (Tn) insertion mutant library in a MDR Pa bloodstream isolate (ID40). The depletion of Tn insertion mutants upon exposure to cefepime or meropenem was measured in order to determine the common resistome for these clinically important anti-pseudomonal  $\beta$ -lactam antibiotics. The approach was validated by clean deletions of genes involved in peptidoglycan synthesis/recycling such as the lytic transglycosylase MltG, the murein endopeptidase MepM1, the MurNAc/GlcNAc-kinase AmgK and the uncharacterized protein YgfB that all were identified in our screen as playing a decisive role for survival of treatment with cefepime or meropenem. We found that the antibiotic resistance of Pa can be overcome by targeting usually non-essential genes that turn essential in the presence of therapeutic concentrations of antibiotics. For all validated genes, we demonstrated that their deletion leads to the reduction of *ampC* expression, resulting in a significant decrease of  $\beta$ -lactamase activity and consequently these mutants partly or completely lost resistance against cephalosporins, carbapenems and acylaminopenicillins. In summary, the determined resistome may comprise promising targets for developing drugs that could be used to restore the sensitivity towards existing antibiotics specifically in MDR strains of Pa. Current studies to unravel the function of YgfB will be discussed.

### 108-MPV

Genome-based analyses of *Klebsiella pneumoniae* to detect possible host- associations, host-adaptation and effects on virulence

\*K. Klaper<sup>1</sup>, L. Heinrich<sup>1</sup>, R. Gerlach<sup>1</sup>, S. Fuchs<sup>1</sup>, Y. Pfeifer<sup>1</sup>, G. Werner<sup>1</sup>

<sup>1</sup>Robert Koch Institut, Infectious Diseases, Wernigerode, Germany

**Introduction:** The increase of "classical" *K. pneumoniae* (cKP) with extended-spectrum- $\beta$ -lactamase production (ESBL-cKP) poses a serious threat to health care. In the last 10 years a new "hypervirulent" but mainly drug-susceptible pathotype of *K. pneumoniae* (hvKP) emerged, associated with community-acquired infections in otherwise healthy individuals. Nowadays, there are more and more reports on antibiotic-resistant hvKP, affecting treatment options in ambulatory care and causing nosocomial outbreaks once introduced into hospitals. Traditionally, hvKP are defined as invasive strains of capsular serotypes K1 and K2 that are string test and *rmpA/A2* positive. However a consensus definition is still missing.

**Objectives:** We investigated genetic characteristics associated with host colonisation or invasion, in order to understand the pathogenicity of hvKP strains.

**Materials and Methods:** A collection of *K. pneumoniae* isolates from different German hospitals, including susceptible cKP, ESBL-cKP and hvKP isolates were subjected to whole genome sequencing (WGS). Phenotypic characterization was done by growth experiments and antibiotic susceptibility assays. To analyse macrophage-mediated phagocytosis, RAW264.7 cells were infected with *K. pneumoniae* isolates and intracellular bacteria were quantified.

**Results:** WGS-based analyses showed that the majority of K1 isolates belonged to the clonal complex CC23, whereas non-K1 isolates were clonally diverse. ESBL and carbapenemases genes were present regardless of the *K. pneumoniae* sequence type and several multidrug-resistant hvKP were detected. Not all isolates harbouring virulence-associated genes displayed a positive string test. Interestingly, *in vitro* phagocytosis assays revealed a lower phagocytosis rate for hvKP than for cKP and ESBL-Kp.

**Conclusion:** Our analyses revealed the emergence of ESBL and carbapenemases-producing hvKP isolates in German hospitals. The string test alone is not suitable to define hvKP; the combination of WGS-based, experimental and clinical data is needed for a reliable identification of hvKP isolates. Further studies are necessary to identify those virulence factors promoting resistance to phagocytosis.

### 109-MPV

Prevalence and diversity of the colibactin-encoding *pks* island in prokaryotes

\*H. Wami<sup>1</sup>, A. Wallenstein<sup>1</sup>, K. Tegelkamp<sup>1</sup>, R. von Buenau<sup>2</sup>, U. Dobrindt<sup>1</sup>

<sup>1</sup>Institute of Hygiene, Universitätsklinikum Münster, Microbial Genome Plasticity, Münster, Germany

<sup>2</sup>Ardeypharm GmbH, Herdecke, Germany

**Introduction:** The bacterial polyketide toxin colibactin is a secondary metabolite that interferes with the eukaryotic cell cycle by causing double-stranded DNA breaks. The genotoxin is discussed to be linked to bacterially induced colorectal cancer in humans. Colibactin is encoded by a

highly conserved, ca. 50-kb genomic (*pks*) island, which is predominantly found in the family *Enterobacteriaceae*, especially *Escherichia coli* and *Klebsiella pneumoniae* isolates from diverse sources and hosts.

**Objectives:** To increase our understanding of the prevalence and diversity of the *pks* island on the genome level.

**Methods:** We performed a genome level study on the *pks* islands found in all publically available prokaryotic genomes (NCBI). We used KAlign for multiple sequence alignment of the unique *pks* islands found in prokaryotes. A phylogenetic tree was generated with RAxML from polymorphisms in core positions of the island.

**Results:** We found that ca. 7% of *E. coli* (mainly phylogroup B2) and *K. pneumoniae*, 40% of *C. koseri*, and 44% of *K. aerogenes* isolates screened harbored the island. The vast majority of *pks* island positive strains belonged to *E. coli* sequence types (ST) 73 and 95 and *K. pneumoniae* ST 258. Various lineages of *pks* islands were found in *Enterobacteriaceae*, which could also be associated with the presence and lineages of the yersiniabactin island, which is closely positioned in the enterobacterial genome.

**Conclusions:** Our data indicate that the *pks* island is stably maintained mainly in enterobacterial species such as *E. coli*, *K. pneumoniae* and *C. koseri*, but can also be found in other *Enterobacteriaceae*. Phylogroup- and even lineage-specific *pks* island variants can be described. Our study provides deeper insights into the evolution, dynamics and structure of the *pks* island in *Enterobacteriaceae*.

## 110-EPV

Elucidating the role of *Candida albicans* Ece1 peptides

\*R. Müller<sup>1</sup>, A. König<sup>1</sup>, D. Yildirim<sup>1</sup>, L. Dally<sup>1</sup>, L. Kasper<sup>1</sup>, S. Mogavero<sup>1</sup>, S. Allert<sup>1</sup>, B. Hube<sup>1,2</sup>

<sup>1</sup>Hans-Knöll-Institut, Microbial Pathogenicity Mechanisms, Jena, Germany

<sup>2</sup>Friedrich Schiller University, Jena, Germany

*Candida albicans* is one of the most common fungal pathogens of humans. It can cause superficial but also life-threatening systemic infections under predisposing conditions. The pathogenicity of *C. albicans* has long been linked to its ability to transition between a unicellular, oval shaped yeast and a filamentous hypha morphology. Conditions that trigger filamentation include, for example, infection of epithelial cells and phagocytosis by macrophages. During hypha formation, the fungus expresses a set of hypha-associated genes, and among those, *ECE1* is one of the most highly expressed. The polyprotein encoded by *ECE1* contains seven recognition sites (KR) for the Golgi-associated endoprotease Kex2. A sequential proteolytic processing of the Ece1 protein at these KR sites produces at least eight individual peptides (Richardson *et al.* 2018). Of the secreted peptides Candidalysin (peptide 3) is the first peptide toxin identified in a human pathogenic fungus, crucial for hypha-associated damage (Moyes *et al.* 2016). Candidalysin is critical for the pathogenicity of both, superficial and systemic infections, as examined in several *in vitro* and *in vivo* infection models. However, the role of the seven other peptides, and their processed versions, is still unknown. Simultaneous expression and secretion of Candidalysin with other Ece1 peptides and a high conservation of these peptide sequences within clinical isolates suggest a crucial function of the non-Candidalysin Ece1 peptides (NCEPs) for *C. albicans*. Analysis using knock out mutants lacking one or several NCEPs and mutants producing Ece1 with modified amino acid sequences of the

peptides suggest that these peptides are essential for normal *C. albicans* hyphal growth and trigger distinct host responses. Further, synthetic versions of NCEPs modulate the action of Candidalysin in an *in vitro* oral epithelial infection model. These data already indicate that NCEPs influence the biology and virulence-associated attributes of *C. albicans*.

Reference: Moyes, D. L. *et al.* *Nature* (2016); Richardson, J. P. *et al.* *mBio* (2018).

## 111-EPV

**Changes in the sterole composition of *Candida glabrata* can be linked to increasing Amphotericin B tolerance**

\*A. M. Aldejohnn<sup>1</sup>, E. Huprich<sup>1</sup>, C. Müller<sup>2</sup>, R. Martin<sup>1</sup>, O. Kurzej<sup>1,3,4</sup>

<sup>1</sup>University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany

<sup>2</sup>Ludwig Maximilians University Munich, Department of Pharmacy, Munich, Germany

<sup>3</sup>Leibniz Institute for Natural Product Research and Infection Biology- Hans Knoell Institute-, Research Group Fungal Septomics, Jena, Germany

<sup>4</sup>Leibniz Institute for Natural Product Research and Infection Biology- Hans Knoell Institute-, National Reference Center for Invasive Fungal Infections, Jena, Germany

## Introduction

*Candida glabrata* acts as a major fungal pathogen often leading to severe bloodstream infections in immunocompromised and critically ill patients. As a haploid organism it has developed various mechanisms of resistance to antifungals like Echinocandins (first line therapy in candidaemia) and Azols. Amphotericin B -a polyene antifungal substance- is the drug of choice in treating multiresistant *Candida glabrata* strains due to a broad susceptibility pattern and little-known resistance as it forms extramembranous aggregates which extract ergosterol out of the fungal cell membrane.

## Objectives

A total number of 115 clinical *C. glabrata* isolates from the NRZmyk strain collection -obtained between 2015 and 2018- were screened for Amphotericin B tolerance. The sterol composition of the fungal cell membrane was analysed in positive found isolates to narrow down dysfunctional enzymes involved in ergosterol biosynthesis, which lead to mutated *ERG* genes.

## Materials and Methods

Screening was performed by microdilution according to EUCAST standards. Sterole analysis was done by MS and compared to a reference strain. Suspected *ERG* genes were amplified by PCR and consequently sequenced.

## Results

A total of 27 strains were found to be tolerant to Amphotericin B (visible growth under 1µg/ml) and eligible for further sterole analysis. Some strains showed an increase of Ergosterol precursor sterols in the fungal cell membrane as a result of defects in enzymes linked to Ergosterol biosynthesis (i.e. C-8 sterol isomerase). A concordant decrease in Ergosterol itself as the major target of polyenes was observed. PCR revealed relevant mutations in the encrypting *ERG* gene family explaining the loss of function. In addition, one strain showed a dysfunctional C5-sterole desaturase



pointing to a mutation in *ERG3* leading to the same decrease in Ergosterol.

## Conclusion

Tolerance to Amphotericin B in *C. glabrata* is still a rare phenomenon. However, our findings indicate that this yeast is able to adapt to and withstand Amphotericin B by alterations in the sterol cell wall composition, exchanging the main target Ergosterol to its non-toxic precursor sterols.

---

## 112-EPV

Living with mold? Decontamination and prevention of fungal growth in manned space flight

\*S. M. Koch<sup>1</sup>, M. Cortesao<sup>1</sup>, C. E. Hellweg<sup>1</sup>, D. Müller<sup>2</sup>, M. Fiebrandt<sup>3</sup>, R. Möller<sup>1</sup>

<sup>1</sup>German Aerospace Center (DLR), Cologne, Institute for Aerospace Medicine, Radiation Biology, Cologne, Germany

<sup>2</sup>Institute of Functional Materials, Material Engineering Center Saarland (MECS), Department of Material Science of Engineering, Saarland, Germany

<sup>3</sup>Institute for Electrical Engineering and Plasma Technology (AEPT), Bochum, Germany

Microorganisms such as archaea, bacteria and filamentous fungi (known as "mold") are an integral part of our human body and our natural environment on Earth. When human space exploration started around 1961, microbial life has been brought along with astronauts to our space stations Mir (Russian Space Station) and ISS (International Space Station).

Filamentous fungi are one of the most abundant pathogens on earth. They emit mycotoxins to our environment, causing allergies and various disease patterns, especially in immunosuppressed humans, which lead to up to over 300 Million fungal infections worldwide. Fungal spores can be a major threat to humans inside a constraint living environment and research addressing this particular health hazard is still underrepresented. The "black mold", is a filamentous fungus called *Aspergillus niger* and one of the main contaminants on the ISS [1, 2]. It is able to spread its spores easily under microgravity and can survive under extreme conditions [3]. Since one of the main sources of fungal infections is due to the uptake of spores via contaminated food or inhalation, a closed built environment like a space station is a major risk factor for astronauts. Due to that, basic research on the resistance of fungal spores towards extreme environments needs to be addressed more. Knowledge about how to effectively constrain and prevent fungal growth in closed environments could not only be applied to human spaceflight but lead to improvements of clean rooms and hospital building materials. In order to find possibilities for efficient decontamination on one hand and targeted preventions against mold on the other hand, investigations of plasma sterilization and antifungal surface development are conducted.

**References:** 1. La Duc and K. Venkateswaran, Microbial monitoring of spacecraft and associated environments. Microb Ecol, 2004 2. Romsdahl, J., et al., Characterization of *Aspergillus niger* isolated from the International Space Station. mSystems, 2018. 3. Moissl-Eichinger, C., et al., Quo vadis? Microbial profiling revealed strong effects of cleanroom maintenance and routes of contamination in indoor environments. Sci Rep, 2015

---

## 113-EPV

Fungal Attack: Chemical Communication and Protection Strategies based on the Secondary Metabolism associated with Tree Canker

\*L. Heck<sup>1</sup>, S. K. Bühring<sup>2</sup>, T. Huber<sup>1</sup>, L. Djurdjevic<sup>1</sup>, L. Antelo<sup>2</sup>, K. Andresen<sup>1</sup>, E. Thines<sup>1,2</sup>

<sup>1</sup>Institute for Molecular Biology, Microbiology and Wine Research, Mainz, Germany

<sup>2</sup>Institute of Biotechnology and Drug Research gGmbH, Kaiserslautern, Germany

*Neovectria ditissima*, the causal agent of tree canker, affects a wide range of hardwood plant hosts like *Fagus*, *Malus* and *Pyrus*. To date, there is no effective method to eradicate the disease and little is known about virulence factors.

In this study we are interested in secondary metabolites as determinants of interaction between the pathogen *N. ditissima* and fungal endophytes within the plant environment. The chemical communication between host and pathogen may give hints for plant protection strategies. Our research is based on the antagonistic and mutualistic balance of the pathogen *N. ditissima* and the endophytic consortia in different host trees. A broad variety of organisms were isolated from healthy plants and these organisms were tested for antagonistic activity against the pathogen. Several compounds were identified from submerged cultures of various endophytic organisms with a protective effect against *N. ditissima*.

During our studies, tree canker inducing metabolites as well as phytotoxins produced by *N. ditissima* were examined. Extracts of the pathogen cultivated in different conditions were tested in an apple assay. Citrinin and p-hydroxybenzoic acid among other substances, were detected. In addition, we were able to identify components with fungicide activity such as llicicolins and ascochlorin were identified.

The genome of *N. ditissima* has been sequenced and potential gene clusters responsible for the secondary metabolite biosynthesis were determined. Moreover, based on transcriptome analysis, we are able to narrow our search of corresponding genes related to phytotoxicity are the target of further studies. In order to assess the function of proteins involved in the biosynthesis of secondary metabolites loss of function mutants are being generated. Another approach is the heterologous expression of the corresponding genes in other fungal hosts.

---

## 114-EPV

Structural redesign of antifungal peptides and analysis of their antifungal activity

\*C. Feurstein<sup>1</sup>, S. Jung<sup>1</sup>, V. Meyer<sup>1</sup>

<sup>1</sup>Technische Universität Berlin, Applied and Molecular Microbiology, Berlin, Germany

Fungal infections lead to estimated 1.6 million deaths in human and destroy one third of crop harvest every year. Antifungal peptides (AFPs), which are produced by fungi represent a strong tool to combat fungal infections in human and plants. AFPs have several characteristics in common including but not limited to a net cationic charge, an amphipathic surface, intramolecular disulfide bridges and predominant  $\beta$ -sheet formation, a  $\gamma$ -core motive as well as high membrane effectiveness.

In this study new chimeric antifungal peptides (cAFP) are designed, whose background are the three naturally occurring antifungal peptides AFP, PAF and AnAFP

produced by *Aspergillus giganteus*, *Penicillium chrysogenum* and *Aspergillus niger* respectively. The new cAFP produced in the heterologous expression system *Pichia pastoris* will be used to perform a susceptibility assay to investigate their antimicrobial activity towards various bacteria, yeast and filamentous fungi.

Additionally, molecular dynamics simulation is performed to further investigate the interaction between the natural and/or chimeric AFPs and the plasma membrane of filamentous fungus and the model organism *A. niger*. Information about the mode of action and specificity gained from these experiments can be used to further improve and redesign AFPs while maintaining their 3D structure, including the  $\beta$ -barrel and the  $\gamma$ -core motive.

Recombination of these peptides and an improved understanding of their mode of action promises to be a powerful approach to tackle multiple pathogens without causing harm to humans, including their bacteriome, or plants.

---

## 115-DKM

### Impact of molecular assays on malaria screening and differentiation

E. Altangerel<sup>1</sup>, U. Loderstädt<sup>2</sup>, E. Tannich<sup>3</sup>, \*H. Frickmann<sup>1,4</sup>

<sup>1</sup>Bundeswehr Hospital Hamburg, Microbiology and Hygiene, Hamburg, Germany

<sup>2</sup>Bernhard Nocht Institute for Tropical Medicine Hamburg, Diagnostic Department, Hamburg, Germany

<sup>3</sup>Bernhard Nocht Institute for Tropical Medicine Hamburg, Head, Hamburg, Germany

<sup>4</sup>University Medicine Rostock, Institute for Medical Microbiology, Rostock, Germany

**Introduction:** Round about 1000 malaria infections per year are imported to Germany, which is hardly enough to maintain high-level routine skills in malaria microscopy outside specialized or reference centers. In European guidelines, less investigator-dependent molecular diagnostic approaches are encouraged for special indications, but the question arises whether they may be generally the better option for screening situations as well as for differentiation on species level.

**Objectives:** The study was performed to assess reliability of different available PCR approaches for primary malaria screening to replace thick blood film assessment.

**Materials & Methods:** In this study, we compared traditional microscopy, three commercial nucleic-acid amplification tests (NAT) for malaria (1 loop-mediated amplification assay, 2 multiplex real-time PCRs) and one in-house real-time multiplex PCR with a collection of 1020 well characterized EDTA blood samples from patients with suspected or confirmed malaria, which were sent for initial or confirmation diagnostics to the German National Reference Center for Tropical Pathogens Bernhard Nocht Institute for Tropical Medicine Hamburg. At least two concordantly positive results were demanded to define a sample as confirmed positive for malaria.

**Results:** Within the 1020 assessed samples, a total of 765 (75%) concordantly negative and 223 (21.9%) concordantly positive results of the four molecular tests were obtained, comprising both samples above and below the microscopic threshold. In contrast, discordant results were observed in 32 (3.1%) instances. For genus-specific assays, detected sensitivity and specificity ranged from 96.4% to 98.4% and from 99.6% to 99.9%, respectively. For species-specific

assays, 94.0%-97.6% sensitivity and 99.6%-100% specificity were found. Falsely negative molecular test results comprised microscopically negative samples, samples at the microscopic detection threshold as well as quantitatively less abundant species in mixed infections.

**Conclusions:** We recorded excellent test characteristics of all assessed molecular assays with only minor differences, suggesting suitability for initial screening.

---

## 116-DKM

### GUARDIAN – Genome based ultrafast detection of bioterrorism agents

\*K. Müller<sup>1</sup>, S. Daßen<sup>2</sup>, J. Stehr<sup>2</sup>, L. Ullerich<sup>2</sup>, F. Bürgens<sup>2</sup>, S. Holowachuk<sup>3</sup>, K. Stoecker<sup>1</sup>

<sup>1</sup>Institut für Mikrobiologie der Bundeswehr, München, Germany

<sup>2</sup>GNA Biosolutions GmbH, Martinsried, Germany

<sup>3</sup>Defence Research and Development Canada Suffield Centre, Suffield, Canada

Molecular diagnostics has become inevitable in the rapid identification of a majority of infectious diseases. In many cases the detection of nucleic acid by polymerase chain reaction (PCR) serves as the gold standard technique for the identification of various infectious agents. It offers many advantages and delivers reliable results with high sensitivity and specificity in one to two hours. However, applicability into the field for rapid on-site diagnostics as it is needed in the investigation of bioterrorist outbreak scenarios is limited. A widely used and portable alternative to PCR are immunochromatographic lateral flow assays (LFAs). They are commercially available and deliver results in just 15 minutes. Unfortunately, they are significantly less sensitive and less specific and can therefore only be used as a rough starting point for further molecular analyses. Here we present an alternative and more sensitive method for the rapid identification of infectious agents using Pulse Controlled Amplification (PCA). PCA is a new class of molecular technology that combines ultrafast wire-based nucleic acid amplification with sample purification and delivers semi-quantitative results in less than 15 minutes. It runs on a small portable battery-powered platform and thus can be taken directly into the field. To test it in the field, we developed a modified assay tailored to our requirements with a focus on fast and easy workflow for performing the PCA wearing our standard NBC protective clothing. Here we show data of our first successful test that we performed during the international CBRN live agent exercise "Precise Response" in 2019 in Suffield, Canada. We were able to detect *Yersinia pestis* via PCA on site in a simulated bioterrorist scenario, without the need of nucleic acid extraction or difficult pipetting steps. Furthermore, we present first results of validation experiments of this newly developed assay along with an outlook into further development and possible applications. In conclusion, PCA holds great potential as a more sensitive and more specific alternative to conventional LFAs for the fast investigation of outbreak scenarios.

---

## 117-DKM

### Sternum osteomyelitis – impact of molecular and microscopic diagnostics

\*J. Kikhney<sup>1</sup>, N. Spindler<sup>2</sup>, A. Wießner<sup>1</sup>, A. Moter<sup>1</sup>

<sup>1</sup>Charité - Universitätsmedizin Berlin, Biofilmzentrum, Institut für Mikrobiologie und Infektionsimmunologie, Berlin, Germany

<sup>2</sup>Universitätsklinikum, Klinik und Poliklinik für Orthopädie, Unfallchirurgie und Plastische Chirurgie, Leipzig, Germany

## Introduction

Mediastinitis, in particular after open-heart surgery, is a devastating infection in a singularly vulnerable patient group.

We questioned if biofilms might play a role in the sternum wounds.

## Objectives

Objectives of this study were to analyze the presence and spatial formation of microorganisms in clinical osteomyelitis samples. Special emphasis was laid on the detection of biofilms in the samples.

## Materials & methods

In a pilot study, we investigated 42 sternum samples from 12 putative osteomyelitis patients from the University clinic of Leipzig by the molecular imaging technique fluorescence in situ hybridization (FISH) in combination with PCR and sequencing. Using FISH, we mapped the presence and formation of microorganisms throughout the samples.

## Results

We found microorganisms by FISH/PCR in all samples investigated, thus confirming the clinical diagnosis of sternum osteomyelitis. In nine cases, we found microscopically single microorganisms that could not be detected by PCR. In three cases, FISH detected single microorganisms, microcolonies and biofilms, respectively. These were identified by PCR (3/3) and FISH (2/3) as *Staphylococcus aureus*, *Enterococcus faecium*, and *S. epidermidis*.

## Conclusion

This pilot study confirmed that FISH/PCR detected microorganisms in mediastinitis samples, shedding light on the formation, location, and invasiveness of the bacteria in the tissue. We were able to interpret the finding of typical skin flora members, thus discriminating between contamination during sampling and infection. Skin flora may cause severe infections and should not be ignored in routine. Out of 12 patients, we found one case featuring mature biofilms. Therefore, mediastinitis is not always associated with biofilms, but microorganisms show a patchy distribution in the tissue.

### 118-DKM

Specific and Rapid Detection of *Bacillus anthracis* Cells by Fluorescent Phage Receptor Binding Fusion Proteins

\*P. Braun<sup>1</sup>, L. Bachstein<sup>1</sup>, A. C. Jacinto<sup>1</sup>, C. Tocantins Santos<sup>1</sup>, G. Grass<sup>1</sup>

<sup>1</sup>Bundeswehr Institute of Microbiology, Munich, Germany

*Bacillus anthracis*, the etiological agent of anthrax disease is typically diagnosed by classical microbiological and molecular methods such as polymerase chain reaction (PCR). Alternatively, mass spectrometry techniques may aid in confirming the presence of the pathogen or its toxins. However, because of the close genetic relationship between *B. anthracis* and other members of the *B. cereus sensu lato* group (such as *B. cereus* or *B. thuringiensis*) mis- or questionable identification frequently occurs in diagnostic laboratories. Bacteriophages such as phage gamma (which is highly specific for *B. anthracis*) have been in use for anthrax diagnostics for many decades. In this work we employed host cell-specific receptor binding proteins of

(pro)phages, also known as tail or head fibers, to develop a microscopy-based approach for the facile, rapid and unambiguous detection of *B. anthracis* cells. For this, the genes of (putative) receptor binding proteins from *Bacillus* phage Wip1, *Bacillus* phage gamma and from lambdoid prophage 03 located on the chromosome of *B. anthracis* were selected. Respective phage genes (Wip1: *gp23* transcriptionally coupled with *gp24*; gamma: *gp14*) and that of lambdoid *B. anthracis* prophage 03 gene *BA4079*) were PCR-amplified and cloned into an expression vector. The phage genes were heterologously expressed in *Escherichia coli* as C-terminal fusions with red fluorescent protein mCherry and an N-terminal twin-strep-tag-epitope for expression verification and purification of the recombinant proteins. *B. anthracis* cells incubated with either of the fusion proteins was successfully surface-labelled in red. Specific labelling of *B. anthracis* was also observed when mixed cultures with other *B. cereus s.l.* species were tested. In contrast, the red fusion proteins did not bind to most cells of a panel of other *B. cereus s.l.* species or to more distantly related bacteria. From these results we anticipate that we can further develop the recombinant receptor binding proteins to become a valuable asset for the detection of *B. anthracis* as a confirmative and rapid means of identification and diagnosis.

### 119-DKM

Prediction of antibiotics resistance in methicillin-resistant *Staphylococcus aureus* from whole genome sequencing data

D. De Coninck<sup>1</sup>, L. Hauben<sup>1</sup>, S. Guedes<sup>2</sup>, \*K. De Rauw<sup>1</sup>

<sup>1</sup>bioMérieux, Clinical Unit, Sint-Martens-Latem, Belgium

<sup>2</sup>bioMérieux, Marcy, France

## Introduction

Widespread and increasing use of antibiotics has driven the emergence of antibiotic resistant bacteria. Methicillin-resistant *Staphylococcus aureus* (MRSA) is of particular concern in health care as it is resistant to commonly used antibiotics and causes worldwide outbreaks. At the same time, whole genome sequencing (WGS) is becoming the method of choice for rapid and accurate identification and characterization of bacteria.

## Objectives

To predict antibiotics resistance in MRSA strains from WGS data.

## Materials & Methods

The BioNumerics *S. aureus* genotyping plugin v0.3 was used to predict antibiotics resistance from genome assemblies of 440 diverse MRSA strains collected from 30 countries (Castillo-Ramirez et al. 2012, Holden et al. 2013, Hsu et al. 2015). The plugin uses a BLAST-based approach with 90% minimum identity and target coverage, both for whole genes and mutations. Starting from the raw sequencing data *de novo* genomes were assembled with Spades v3.7.1 in BioNumerics v7.6.3. Predictions of the plugin were compared to classically obtained AST results to calculate specificity and sensitivity of the predictions.

## Results

Predictions of 15 antibiotics (ciprofloxacin, clindamycin, daptomycin, erythromycin, fosfomycin, fusidic acid, gentamycin, linezolid, tetracycline, mupirocin, oxacillin,

penicillin, rifampicin, teicoplanin and vancomycin) were compared to the classically obtained AST data. Sensitivity ranged between 0 and 1 (median = 0.21). A sensitivity of 0 was only obtained for 6 antibiotics for which no positive data was present in the dataset. For these antibiotics, true negatives were obtained for 98 to 99.5% of the cases. Specificity ranged from 0.61 to 1 (median = 0.99). For 12 of the 15 antibiotics a specificity higher than 0.95 was obtained. Lowest specificity (0.61) was obtained for the antibiotics ciprofloxacin and clindamycin. For penicillin a specificity of 0.83 was observed.

## Conclusion

Based on WGS data, fairly accurate predictions of antibiotics resistance in MRSA strains can be obtained. Yet, profound knowledge of resistance mechanisms to some antibiotics is lacking, leading to less reliable predictions.

## 120-DKM

### Proteotyping: Proteomics- and Genomics-Based Diagnostics of Infectious Bacteria, Virulence and Anti-Microbial Resistance

\*E. R. B. Moore<sup>1,2,3</sup>, F. Salvà-Serra<sup>1,2,3,4</sup>, D. Jaén-Luchoro<sup>1,3</sup>, R. Karlsson<sup>1,2,3,5</sup>

<sup>1</sup>University of Gothenburg, Department of Infectious Diseases, Gothenburg, Sweden

<sup>2</sup>Sahlgrenska University Hospital, Department of Clinical Microbiology, Gothenburg, Sweden

<sup>3</sup>University of Gothenburg, Centre for Antibiotic Resistance Research (CARE), Gothenburg, Sweden

<sup>4</sup>University of the Balearic Islands, Department of Biological Sciences, Palma de Mallorca, Spain

<sup>5</sup>Nanoxis Consulting AB, Gothenburg, Sweden

The global expansion of bacteria manifesting anti-microbial resistance (AMR) presents challenges for treatment and prevention; the World Health Organisation (WHO) has predicted an overwhelming impact of diseases for which no antibiotic treatment will be available [1]. With escalation of AMR, combined with continuing decline in new antibiotic discovery, development of innovative, reliable, rapid and cost-efficient analytical techniques for effective diagnostics of infectious disease is increasingly essential to confront rising mortality and costs associated with AMR infections. However, the routine methodologies used today for diagnosing infectious disease depend, in most cases, upon multiple protocols requiring cultivation of the pathogenic bacteria from clinical samples. With accumulating whole-genome sequence data becoming available, Mass Spectrometry (MS)-based proteomics analyses increasingly are able to be applied to biological studies. Proteomic analyses are indirect analyses of genomes; the proteome comprises proteins from the expressed genes of a cell, organism or biological system. Proteotyping [2], using state-of-the-art LC-MS/MS analyses of generated cellular peptides, enables detection and identification of bacterial species, virulence- and AMR-factors from single MS analyses. In this study, reference strains of clinically-relevant bacteria were analysed by proteotyping, to define the reliable peptide biomarkers that could be used for diagnostics of infectious diseases (i.e., discovery). The biomarker peptides were applied for discrimination of bacterial species and detection of known virulence- and AMR-factors, *in vitro*, and also for detection and diagnostics of infective bacteria, without prior cultivation, *in vivo* (i.e., validation). Notably, comprehensive and accurate genome sequence data is key to resolving peptide matching and protein discrimination for identification and characterisation of infective bacteria and infectious diseases.

## References

[1] World Health Organization. 2014. Antimicrobial Resistance: Global Report on Surveillance. ISBN: 978-92-4-15674-8.

[2] Karlsson et al. 2015. Syst Appl Microbiol 38:246-257.

## 121-DKM

A host response mRNA signature from blood accurately diagnoses and prognoses acute infections and sepsis in emergency department patients

W. Bauer<sup>1</sup>, K. Kappert<sup>2</sup>, N. Galtung<sup>1</sup>, J. Wacker<sup>3</sup>, \*O. Liesenfeld<sup>3</sup>, R. Tauber<sup>2</sup>, R. Somasundaram<sup>1</sup>

<sup>1</sup>Charité Universitätsmedizin Berlin, Emergency and Acute Medicine, Berlin, Germany

<sup>2</sup>Charité Universitätsmedizin Berlin, Institute of Laboratory Medicine, Clinical Chemistry and Pathobiochemistry, Berlin, Germany

<sup>3</sup>Inflammatix Inc, Clinical Affairs, Burlingame, CA, United States

Rapid diagnostic tests for the detection of acute bacterial and/or viral infection and sepsis are needed in the emergency department (ED). Current diagnostic and prognostic tests are impeded, e.g. by slow turnaround time for blood cultures. Differentiating between bacterial and viral infections as well as managing the appropriate level of care are critical deliverables. Thus, over-treatment leading to antimicrobial resistance, as well as *C. difficile* infection or late diagnosis and treatment of sepsis may result. In a prospective single-site study at the ED of the Charité University Hospital (Campus Benjamin Franklin) we investigated the efficacy of a novel host response test from blood which uses 29 host response mRNA targets and a machine learning algorithm (HostDx Sepsis) to quickly and accurately differentiate between viral and bacterial infections including co-infections and non-infected patients and predict the probable clinical course. Patients who presented with signs of acute infection were enrolled. All patients were adjudicated using chart review by two expert physicians using clinical data, radiological and laboratory tests (incl. C-reactive protein (CRP) and procalcitonin (PCT)) but blinded to the HostDx Sepsis results. We here present an interim analysis of 125 patients enrolled and adjudicated since January 2019. The accuracy of HostDx Sepsis predictions was compared to standards of rapid bacterial infection detection, such as CRP and PCT. The Area Under the Receiver Operating Characteristics (AUROC) for HostDx Sepsis at predicting bacterial and co-infections vs. non-infected and virally infected patients was 0.89 compared to 0.87 for PCT and 0.83 for CRP. HostDx Sepsis also predicted viral and co-infections vs. non-infection and bacterial infection with an AUROC of 0.85. When combined with Sequential Organ Failure Assessment (SOFA)-scores the test predicted multiorgan failure with an AUROC of 0.89, compared to 0.86 for SOFA-scores alone. Our results suggest that the use of a host-response test to determine the infection status and severity allows for more informed rapid decisions on antibiotic treatment and further level of care in the ED.

## 122-DKM

Improved microbiological identification and antimicrobial resistance testing of clinical *Nocardia ssp.* isolates by 16s rRNA analysis and whole genome sequencing

\*P. Chhatwal<sup>1</sup>, S. Woltemate<sup>1</sup>, T. Welte<sup>2</sup>, S. Ziesing<sup>1</sup>, D. Schlüter<sup>1</sup>, M. Vital<sup>1</sup>

<sup>1</sup>Hannover Medical School (MHH), Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany

## Introduction

Nocardiosis is a rare but life threatening infection caused by aerobic *Actinomycetes* of the genus *Nocardia* particularly affecting immunocompromised hosts. The identification of *Nocardia* ssp. and antibiotic susceptibility testing (AST) by standard microbiological methods may be incomplete but molecular techniques may improve microbiological diagnostics.

## Objectives

To determine phylogenetics and antimicrobial resistance mechanisms of clinical *Nocardia* isolates, we characterized *Nocardia* strains from patient material using different methods for taxonomic identification and AST as well as whole genome sequencing (WGS).

## Materials & methods

We studied *Nocardia* strains isolated from patient material between 2000 and 2018. For taxonomic identification we applied different MALDI-TOF (bioMérieux, Saramis database) protocols and 16S rRNA gene-sequencing. Antibiotic resistance was tested based on a microdilution method according to the CLSI breakpoints and compared with Etests. WGS was performed using the Illumina workflow.

## Results

We examined 40 *Nocardia* strains from 33 patients. Twenty-four patients (72.7%) were immunocompromised due to solid organ transplantation or stem cell transplantation. MALDI-TOF analysis correctly identified 31 isolates (77.5%). An advanced MALDI-TOF protocol with formic acid and acetonitrile treatment increased identification to 37 isolates (92.5%). One *N. amamiensis* strains was misidentified by MALDI-TOF as *N. abscessus* as revealed by molecular analysis. Two additional isolates were identified as *N. lasii* via molecular techniques. AST by Etest and microdilution, respectively, showed good concordance with an overall trimethoprim-sulfamethoxazole (SXT) resistance rate of 22.2%. WGS of a SXT resistant *N. farcinica* isolate showed a deletion of several amino acids compared to SXT sensitive *N. farcinica* isolates (n=10) in a homolog of dihydropteroate synthase.

## Conclusion

A combination of different biochemical and molecular methods for taxonomic identification and AST improved species identification and AST results compared to standard microbiological techniques. WGS revealed molecular insights into SXT resistance mechanisms.

### 123-MCBV

Stalk-terminal budding in *Hyphomonas neptunium*

proceeds via an FtsZ-driven process involving two spatially distinct divisome-like complexes

\*M. van Teeseling<sup>1</sup>, S. Eiseheuer<sup>1,2</sup>, J. Zimmer<sup>1,2</sup>, M. Thanbichler<sup>1,2,3</sup>

<sup>1</sup>Philipps Universität Marburg, AG Thanbichler, Marburg, Germany

<sup>2</sup>Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

<sup>3</sup>LOEWE Center for Synthetic Microbiology, Marburg, Germany

Many well-studied bacteria divide via binary fission and although several bacteria provide offspring in alternative ways, the mechanisms underlying these alternative modes of cell division remain mostly unknown. In stalk-terminal budding, cells grow a cytoplasm-filled stalk from their cell body, and the tip of the stalk then develops into a bud. This mode of cell division is highly asymmetric, as the constriction exclusively takes place at the budneck, giving rise to an ovococoid daughter cell and a stalked mother cell. Stalk-terminal budding occurs in several alphaproteobacterial species, including the newly established model organism *Hyphomonas neptunium*.

Here, we investigate the mechanism of stalk-terminal budding. We show that this process relies on FtsZ. Strikingly, FtsZ localizes not only to the future division site, but also to the stalk base. Comprehensive localization studies reveal that the majority of the canonical divisome components localize exclusively to the future division site, whereas a subset of them also colocalizes with FtsZ at the stalk base. Moreover, we show that one of the canonical divisome components has adapted a novel function in stalk-terminal budding. These results suggest that stalk-terminal budding requires adaptations in both the localization, and possibly the function, of the canonical cell division machinery.

---

### 124-MCBV

New flat embedding method for transmission electron microscopy reveals an unknown mechanism of tetracycline

\*M. Wenzel<sup>1</sup>, M. P. Dekker<sup>2</sup>, B. Wang<sup>3</sup>, M. J. Burggraaf<sup>2</sup>, W. Bitter<sup>2</sup>, J. R. T. van Weering<sup>2</sup>, L. W. Hamoen<sup>3</sup>

<sup>1</sup>Chalmers University of Technology, Göteborg, Sweden

<sup>2</sup>Amsterdam University Medical Centers, Location VUMC, Amsterdam, Netherlands

<sup>3</sup>University of Amsterdam, Amsterdam, Netherlands

Transmission electron microscopy (TEM) is an important imaging technique in bacterial research and requires ultrathin sectioning of resin embedding of cell pellets. This method consumes milli- to deciliters of culture and results in sections of randomly orientated cells. For rod-shaped bacteria, this makes it exceedingly difficult to find longitudinally cut cells, which precludes large-scale quantification of morphological phenotypes. Here, we describe a new fixation method using either thin agarose layers or carbon-coated glass surfaces that enables flat embedding of bacteria. This technique allows for the observation of thousands of longitudinally cut rod-shaped cells per single section and requires only microliter culture volumes. We successfully applied this technique to Gram-positive *Bacillus subtilis*, Gram-negative *Escherichia coli*, the tuberculosis vaccine strain *Mycobacterium bovis* BCG, and the cell wall-lacking mycoplasma *Acholeplasma laidlawii*. To assess the potential of the technique to quantify morphological phenotypes, we examined cellular changes induced by a panel of different antibiotics. Surprisingly, we found that the ribosome inhibitor tetracycline causes significant deformations of the cell membrane. Further investigations showed that the presence of tetracycline in the cell membrane changes membrane organization and affects the peripheral membrane proteins MinD, MinC, and MreB, which are important for regulation of cell division and elongation. Importantly, we could show that this effect is not the result of ribosome inhibition but is a secondary antibacterial activity of tetracycline that has defied discovery for more than 50 years.

---

### 125-MCBV

Predicting membrane lipid composition from *in silico* data – how far can we go?

\*B. J. Tindall<sup>1</sup>

<sup>1</sup>Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Braunschweig, Germany

<sup>8</sup>IOW, Warnemünde, Germany

<sup>9</sup>HZI Braunschweig, Braunschweig, Germany

Cell membranes are the most complex "organ" of the cell, with up to 50% of a cell's genome encoding components that are associated with the membrane. A key step in the formation of modern membranes is the evolution of the classical lipid mediated "bilayer" in the form of a hydrophilic-hydrophobic-hydrophilic sandwich. Complex lipid-lipid and lipid-protein interactions, based on the chemical and physical properties of the interacting partners determine a membrane's integrity and whether it is compromised, that would lead to the death of the cell. As an ever increasing number of genomes become available the question arises to what degree can one predict lipid contribution, based on genomic data, to the structure and functioning of the cell.

## 126-MCBV

### Formation of magnetic organelles

\*R. Uebe<sup>1</sup>

<sup>1</sup>Universität Bayreuth, LS Mikrobiologie, Bayreuth, Germany

The biogenesis and assembly of membranous cell organelles requires a multitude of processes, including membrane remodeling and correct spatio-temporal targeting of proteins. These processes are relatively well characterized in eukaryotes and were shown to involve membrane bending proteins, vesicular transport and/or organelle-specific protein translocation systems. In contrast, the biogenesis and assembly of prokaryotic organelles, which usually do not have the same complexity as their eukaryotic counterparts, often remained poorly understood.

Here we analyzed the formation of magnetosomes, membrane-enclosed magnetite nanoparticles used for magnetic navigation, as a model system for prokaryotic organelle formation. Genetic analyses in *Magnetospirillum gryphiswaldense* MSR-1 revealed that magnetosome formation is complex process that is governed by specific magnetosome-associated proteins. MamB, a cation diffusion facilitator (CDF) family member, for example, has been implicated in magnetosome-directed iron transport. However, deletion mutagenesis studies revealed that MamB is essential for the formation of magnetosome membrane vesicles. Using site-directed mutagenesis complemented by structural analyses, fluorescence microscopy and cryo-electron tomography, we show that MamB is most likely an active magnetosome-directed transporter serving two distinct, yet essential functions. First, MamB initiates magnetosome vesicle formation in a transport-independent process, probably by serving as a landmark protein. Second, MamB transport activity is required for magnetite nucleation.

## 127-MCBV

The novel shapeshifting bacterial phylum *Saltatorellota*

\*S. Wiegand<sup>1,2</sup>, M. Jogler<sup>3</sup>, T. Kohn<sup>1</sup>, R. P. Awal<sup>4</sup>, S. Oberbeckmann<sup>5</sup>, K. Kesý<sup>5</sup>, O. Jeske<sup>6</sup>, P. Schuhmann<sup>6</sup>, S. H. Peeters<sup>1</sup>, N. Kallscheuer<sup>1</sup>, M. Strauss<sup>7</sup>, A. Heuer<sup>6</sup>, M. S. M. Jetten<sup>1</sup>, M. Labrenz<sup>8</sup>, A. K. Kaster<sup>2</sup>, M. Rohde<sup>9</sup>, C. Boedeker<sup>6</sup>, H. Engelhardt<sup>7</sup>, D. Schüler<sup>4</sup>, C. Jogler<sup>1,3</sup>

<sup>1</sup>Radboud Universiteit Nijmegen, Microbiology, Nijmegen, Netherlands

<sup>2</sup>Karlsruhe Institute of Technology, Karlsruhe, Germany

<sup>3</sup>Friedrich-Schiller-Universität Jena, Jena, Germany

<sup>4</sup>University Bayreuth, Bayreuth, Germany

<sup>5</sup>IOW, Warnemünde, Germany

<sup>6</sup>DSMZ, Braunschweig, Germany

<sup>7</sup>Max Planck Institute of Biochemistry, Martinsried, Germany

Our current understanding of a free-living bacterium - capable of withstanding a variety of environmental stresses - is represented by the image of a peptidoglycan-armored rigid casket. The making and breaking of peptidoglycan greatly determines cell shape. The cytoplasmic membrane follows this shape, pressed towards the cell wall by turgor pressure. Consequently, bacteria are morphologically static organisms, in contrast to eukaryotic cells that can facilitate shape changes. This concept of a bacterial cell is challenged by the discovery of the novel bacterial phylum *Saltatorellota*. Members of this phylum can change their shape, are capable of amoeba-like locomotion and trunk-formation through the creation of extensive pseudopodia-like structures. Two independent *Saltatorellota* cells can fuse, and they employ various forms of cell division from budding to canonical binary fission. Despite their polymorphisms, members of the *Saltatorellota* do possess a peptidoglycan cell wall. Their genomes encode flagella and type IV pili as well as a bacterial actin homolog, the "saltatorellin". This protein is most similar to MamK, a dynamic filament-forming protein, that aligns and segregates magnetosome organelles via treadmilling. We found saltatorellin to form filaments in both, *E. coli* and *Magnetospirillum gryphiswaldense*, leading to the hypothesis that shapeshifting and pseudopodia formation might be driven by treadmilling of saltatorellin. Furthermore, some unusual traits of *Saltatorellota* strains seem to be occurring only in a small fraction of newly formed daughter cells, implying some kind of cell differentiation.

## 128-MCBV

### Analysis of the *Clostridioides (Clostridium) difficile* motility behaviour by video microscopy revealed a novel swimming pattern

\*J. Schwanbeck<sup>1</sup>, I. Oehming<sup>1</sup>, A. E. Zautner<sup>1</sup>, U. Groß<sup>1</sup>, W. Böhne<sup>1</sup>

<sup>1</sup>Universitätsmedizin Göttingen, Institut für Medizinische Mikrobiologie, Göttingen, Germany

### Introduction:

Most strains of the gastrointestinal pathogen *C. difficile* produce peritrichous flagella, which mediate swimming motility. Motility is most often examined in soft agar based assays. However, little knowledge exists on the actual swimming behaviour of single *C. difficile* cells.

### Objectives:

We wondered if direct observation of swimming motility via video microscopy could offer more insights into the motility patterns of *C. difficile*.

### Materials & Methods:

We designed an open-source python program that allows us to simultaneously track a large number of bacterial cells on standard computers. With the aid of this program it was possible to determine and quantify the most relevant *C. difficile* motility parameters, as travelled distance, speed, percentage of time where bacteria are motile and turning points.

### Results:

The *C. difficile* 630Δ*erm* and *C. difficile* R20291 populations were found to consist of three fractions including (i) non-motile bacteria, (ii) bacteria with a high frequency twitching

pattern, and (iii) bacteria with long run phases. The size of the individual fractions is variable and depends on the nutrient conditions. The fraction with long run phases appears to be able to swim forward and backwards without an obvious preference. This is in contrast to the usual motility pattern of bacteria, which consists of a preferred direction of travel coupled with a reorientation phase.

## Conclusions:

We found that *C. difficile* displays a swimming behaviour that appears to be different from known swimming patterns of bacteria described in literature.

## Acknowledgment:

This work was funded by the Federal State of Lower Saxony, Niedersächsisches Vorab (VWZN2889/3215/3266.)

## 129-MCBV

### Caught in the act: pervasive transcription during the low-energy phase of the budding yeast growth cycle.

\*R. Machne<sup>1</sup>, S. Bernhard<sup>2</sup>, I. M. Axmann<sup>1</sup>, P. F. Stadler<sup>2</sup>, D. B. Murray<sup>3</sup>

<sup>1</sup>HHU Duesseldorf, Synthetic Microbiology, Duesseldorf, Germany

<sup>2</sup>Universität Leipzig, Interdisciplinary Center for Bioinformatics, Leipzig, Germany

<sup>3</sup>Keio University, IAB Tsuruoka, Tsuruoka, Japan

When grown in continuous culture at high cell density budding yeast cells autonomously synchronize their metabolic activity into stable oscillations, and these metabolic cycles percolate throughout physiology and gate cell division cycle check-points. Metabolic cycles can persist even in non-dividing yeast cells. The industrial diploid strain IFO 0233 shows a strong decoupling from division, with metabolic cycles about 10x faster than doubling times. Despite the diversity of metabolic dynamics between strains, cycling cultures traverse a conserved yet adaptable transcriptional program, between the expression of growth and anabolic, and catabolic and stress-response functions, respectively. The dynamics of non-coding transcription have not yet been explored.

An RNA-seq timeseries was sampled in high temporal resolution from a chemostat culture during a metabolic transient of the oscillation. The temporal program of coding transcripts encodes the spatial structure of the growing cells and is itself organized into large chromosomal domains of co-expressed clusters. This spatio-temporal organization may support co-translational complex formation and optimal proteome resource allocation. An unprecedented level of non-coding transcription from loci involved in auto-editing of the yeast genome (rDNA, mating type, CUP1) and evolution of gene regulation (transposons) indicates a recombination-prone state during the G1-like

low-energy phase of the metabolic cycle. Genetically dynamic loci, tRNA, replication origins, and free-standing "long terminal repeats" are all enriched at the borders between high-energy and low-energy phase transcription domains. Our data provides a snap-shot of the spatial and functional constraints of genome evolution, and suggests a role of "pervasive transcription" in adaptive exploration of novel phenotypes.

## 130-MCBV

### The uncharacterized DUF1127 protein from *Rhodobacter sphaeroides* binds RNA

\*J. Grützner<sup>1</sup>, G. Klug<sup>1</sup>

<sup>1</sup>Institute of Microbiology and Molecular Biology, University of Giessen, Gießen, Germany

The facultative phototrophic bacterium *Rhodobacter sphaeroides* can adapt its life style to changing environmental conditions. In the past our group elucidated in *R. sphaeroides* the role of protein regulators and small, regulatory RNAs (sRNAs) in stress responses, particularly to oxidative stress. The four homologous sRNAs CcsR1-4 (conserved CCUCCUCCC motif stress-induced RNA) are expressed under various stress conditions and modulate the C1 metabolism [1]. These sRNAs are derived from the 3' UTR of the RSP\_6037 mRNA. The RSP\_6037 gene encodes a small protein of 70 amino acids and the residues 23-62 constitute a domain of unknown function (DUF1127). This functional unknown protein domain is structurally related to eukaryotic RNA binding proteins [2]. Small proteins with the DUF1127 domain are widely distributed among Alpha- and Gammaproteobacteria and more than 11000 bacterial sequences with DUF1127 proteins are listed in InterPro.

Our data show that the small protein RSP\_6037 influences the amount of the CcsR sRNAs and that the uncharacterized DUF1127 domain is sufficient for the function. By co-immunoprecipitation we could show binding between the small DUF1127 protein, the sRNA CcsR1 and other RNA transcripts. We test the hypothesis that binding of the DUF1127 domain of RSP\_6037 influences the stability and processing of RNA transcripts and may play an important role in RNA maturation.

[1] Berghoff BA, Glaeser J, Sharma CM, Vogel J, Klug G. (2009) Photooxidative stress-induced and abundant small RNAs in *Rhodobacter sphaeroides*. *Mol Microbiol.* **74**: 1497-512.

[2] Billenkamp F, Peng T, Berghoff BA, Klug G (2015) A cluster of four homologous small RNAs modulates C1 metabolism and the pyruvate dehydrogenase complex in *Rhodobacter sphaeroides* under various stress conditions. *J. Bacteriol.* **197**: 1839-1852

## 131-MSHV

### Microbiota Changes in Primary Sclerosing Cholangitis

\*C. Bang<sup>1</sup>, M. C. Rühlemann<sup>1</sup>, T. Liwinski<sup>2</sup>, R. Zenouzi<sup>2</sup>, M. Kummer<sup>3</sup>, W. Lieb<sup>4</sup>, J. R. Hov<sup>3</sup>, C. Schramm<sup>2</sup>, A. Franke<sup>1</sup>

<sup>1</sup>Christian-Albrechts-University Kiel, Institute of Clinical Molecular Biology, Kiel, Germany

<sup>2</sup>I. Department of Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

<sup>3</sup>Norwegian PSC Research Center, Division of Surgery,



## Question

While the aetiology of primary sclerosing cholangitis (PSC) remains largely unknown, several reports, including our own previous data, support a functional and potentially pathogenic link between the intestinal microbiota and liver inflammation in PSC. However, it is unclear whether the microbial signature so far described in PSC single-centre cohorts is centre-specific or if a PSC-specific microbial signature across different geographical regions exists.

## Methods

We included in total 388 individuals from two separate cohorts from Norway and Germany: 137 PSC patients (n=75 with concurrent colitis), 88 patients with ulcerative colitis (UC) and 133 healthy controls (HC). The faecal microbiota was analysed by 16S rRNA gene sequencing (V1-V2). In addition, stool samples from individuals of the German cohort (66 HC, 65 PSC patients and 38 UC patients) was subjected to ITS2 gene sequencing to unravel alterations in the mycobiome.

## Results

In both cohorts, the global microbiota composition (beta-diversity) was significantly different when comparing PSC and HC ( $P < 0.001$ ). Multiple robust single microbial markers of PSC were identified, including novel associations with Proteobacteria and the bile tolerant genus *Parabacteroides*, and previous associations with e.g. *Veillonella* and *Lactobacillus*. Within the mycobiome we found a significant increase of *Trichocladium griseum* belonging to the class Sordariomycetes as well as of *Candida* species in stool samples of PSC patients confirming earlier published results within a French cohort<sup>1</sup> at increased taxonomic resolution.

## Conclusions

Patients with PSC display reproducible microbiota alterations compared to HC, independent from geographical region and concurrent colitis, which could relate to PSC itself and be of clinical relevance.

<sup>1</sup>Lemoinne S, Kemgang A, Ben Belkacem K, et al. Fungi participate in the dysbiosis of gut microbiota in patients with primary sclerosing cholangitis. *Gut* 2019 doi: 10.1136/gutjnl-2018-317791

### 132-MSHV

Spore formers are initial butyrate producers during infant gut microbiota development

\*C. Schwab<sup>1,2</sup>, O. Appert<sup>2</sup>, A. Ramirez Garcia<sup>2</sup>, R. Ferstl<sup>3</sup>, E. Bersuch<sup>3</sup>, S. Loeliger<sup>3</sup>, C. Lacroix<sup>2</sup>, R. Lauener<sup>4</sup>, R. Frei<sup>3</sup>, C. Roduit<sup>5</sup>

<sup>1</sup>Czech University of Life Sciences, Prag, Czech Republic

<sup>2</sup>ETH Zürich, Zurich, Germany

<sup>3</sup>Christine Kühne - Center for Allergy Research and Education (CK-CARE), Davos, Switzerland

<sup>4</sup>Kinderspital St. Gallen, St. Gallen, Germany

<sup>5</sup>Kinderspital Zurich, Zurich, Switzerland

The acquisition and microbial succession of the infant gut microbiota are key to establishing a host-microbiota symbiosis. The early gut microbiota provides environmental conditions for later colonization through the formation of

short chain fatty acids (SCFA). Spores might be a source of environmental transition.

We were interested in the role of spore formers in early microbiota colonization, and their impact on butyrate production. We used a combined cultivation-dependent and -independent approach to determine cultivable spores, spore and fecal microbiota composition and SCFA formation in a cohort of infants (n=69, CARE-Childhood, AlleRgy, Nutrition and Environment, Switzerland) at 90, 180 and 360 days of age.

At 90 days, about 80% of the samples contained cultivable spores at a mean most probable number of log 3.4 cells/g. Cultivable spore prevalence and abundance was significantly higher in feces from 360 days (100% and log 4.2 cells/g, respectively) and compared to 180 and 90 days. Butyrate was produced by 50, 78, and 94% of the spore samples during germination at 90, 180 and 360 days.

The cultivable spore microbiota was dominated by *Clostridiaceae* (mean abundance 50-77%). *Bifidobacteriobacteriaceae* (mean abundance 90 days: 66%, 180 days 55%, 360 days 31%) were the major bacterial family in feces with *Clostridiaceae* contributing 1-5% of the reads. Nevertheless, the spore forming *Clostridium* cluster I was the most prevalent and abundant butyrate producing group at 90 days. Abundance ranged from log 6.3 to log 8.5 cells/g in approximately 40 % of the samples. Abundance of non-spore forming butyrate producers *Faecalibacterium prausnitzii*, *Eubacterium rectale* / *Roseburia* spp. and *Eubacterium hallii* was low at 90 days ( $< \log 5$  cells/g) but significantly increased at 360 days together with mean fecal butyrate levels (1.7 mM/g at 90 days and 12.6 mM/g at 360 days).

Our data show the impact of spore forming butyrate producers on SCFA formation especially at the early stages of infant gut microbiota development. Our data may suggest opportunities to enhance early life butyrate concentrations through the supplementation of selected groups of butyrate producers.

### 133-MSHV

Exploring novel small proteins in a simplified human gut microbiome

\*H. Petruschke<sup>1</sup>, C. Schori<sup>2,3</sup>, C. H. Ahrens<sup>2,3</sup>, N. Jehmlich<sup>1</sup>, M. von Bergen<sup>1,4</sup>

<sup>1</sup>UFZ, MOLSYB, Leipzig, Germany

<sup>2</sup>Agroscope, Molecular Diagnostics, Genomics & Bioinformatics, Wädenswil, Switzerland

<sup>3</sup>SIB Swiss Institute of Bioinformatics, Wädenswil, Switzerland

<sup>4</sup>University of Leipzig, Institute of Biochemistry, Leipzig, Germany

Hannes Petruschke<sup>1</sup>, Christian Schori<sup>2</sup>, Christian H. Ahrens<sup>2</sup>, Nico Jehmlich<sup>1</sup>, Martin von Bergen<sup>1,3</sup>

<sup>1</sup>Helmholtz-Centre for Environmental Research - UFZ, Department of Molecular Systems Biology, Permoserstr. 15, 04318 Leipzig, Germany; <sup>2</sup>Agroscope, Molecular Diagnostics, Genomics & Bioinformatics and SIB Swiss Institute of Bioinformatics, Wädenswil, Switzerland; <sup>3</sup>Institute of Biochemistry, Faculty of Biosciences, Pharmacy and Psychology, University of Leipzig, Germany

The intestinal microbiota plays a pivotal role in protecting the host against pathogenic microbes, modulating immunity and regulating metabolic processes. We are using a simplified human microbial community of 8 species for the investigation of metabolic output and species interdependence. The

objective of this study is the detection of novel proteins smaller than 100 amino acids (= sProteins) that are potentially regulating the simplified intestinal human microbiota. sProteins have largely been unrecognized and little is known about their structure and function in microbes. Several studies suggested that sProteins have a wide range of functions including significant structural re-organization of microbial communities.

In this study, we applied different sProtein enrichment strategies (e.g. C8-cartridges and GelFree fractionation) before mass spectrometric analysis since most sProteins can not be identified using standard protocols. In addition, after sequencing, de novo assembly and annotation of the complete genomes of the 8 species, we created an integrated proteogenomics search database (iPtgxDB). Searching MS/MS data against the iPtgxDB, we were able to identify several novel sProteins which will be further validated using metatranscriptome analysis. Notably, several novel sProteins were not identified in single strain cultivations possibly indicating a fundamental role in microbial community organization.

Identification and the further functional characterization of sProteins will change the view on microbial community organization.

---

### 134-MSHV

Exploring pathogenic functions of host microbiota

\*M. Vital<sup>1</sup>, S. Rath<sup>2</sup>, D. Pieper<sup>2</sup>, D. Schlüter<sup>1</sup>

<sup>1</sup>Hannover Medical School, Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

It is becoming evident that certain features of human microbiota, encoded on distinct taxa including autochthonous members, promote disease. As a result, borders between so-called opportunistic pathogens, pathobionts and commensals are increasingly blurred and specific, organism(bacterial)-centric targets for manipulating microbiota to improve host health are becoming elusive. In this study, we focus on functions of bacterial communities that have the potential to cause disease, proposing the term "pathogenic functions (pathofunctions)". The concept is presented via three distinct examples, namely, the formation of (i) trimethylamine, (ii) secondary bile acids and (iii) hydrogen sulfide that all represent metabolites of gut microbiota linked to the development of non-communicable diseases. Using publicly available metagenomic and metatranscriptomic data (n=2,975) we quantified those pathofunctions in health and disease and exposed the key players. Pathofunctions were ubiquitously present, even in healthy microbiota, though increased abundances were detected in patient groups. Overall, the three pathofunctions were measured at low mean concentrations (<1% of total bacteria) and encompassed various taxa, including uncultured members. We outline how this function-centric approach, where all members exhibiting a particular pathofunction are redundant, guides risk assessment and can assist the development of precision treatment directing gut microbiota to increase host health.

---

### 135-MSHV

Single-Cell Dispensing for High-Throughput Bacterial Isolation

\*A. Afrizal<sup>1</sup>, A. Viehof<sup>1</sup>, T. Hitch<sup>1</sup>, T. Clavel<sup>1</sup>

<sup>1</sup>RWTH Aachen University Hospital, Institute of Medical Microbiology, Aachen, Germany

The intestinal microbiota of mammals is known to influence their health, however a large proportion of bacterial species in gut communities are still unknown. Cultivation is essential to improve genome databases and to explore the functional potential of bacteria. Agar plate-based approaches are still widely used for bacterial isolation, but are time-consuming. The use of single cell dispensers (SCD) allow high-throughput, label-free bacterial sorting. This project aimed to study the feasibility of using SCD for the isolation of gut bacteria and to design a more efficient workflow for bacterial cultivation.

The initial feasibility study was conducted using pure cultures and enriched faecal samples in both aerobic and anaerobic conditions. To increase throughput, 96-well plates were used for cell dispensing. Enrichment trials were also tested for their ability to generate varying input bacterial compositions upstream of sorting to maximize the harvested cultured diversity.

SCD was able to sort single cells from two different pure cultures of strictly anaerobic gut bacteria at an average efficacy (percentage of sorted single-cells giving rise to growth) of 7% (motile bacteria) and 69% (non-motile bacteria). Direct processing of a frozen glycerol stock of mouse faecal sample resulted in low efficacy (ca. 4%) when compared with enriched mouse faecal cultures (ca. 37-87%), but recovered diversity of isolates was higher (9 different species out of 24 isolates tested vs. 18 of 1,356). Two novel bacterial species were cultured from a human faecal sample (*Bacillus* sp. nov.) and a mouse caecal sample (*Flintibacter* sp. nov.) using the new SCD approach. Out of the media tested so far, 12/15 enriched on average 54% of dominant ( $\geq 1\%$  rel. Abund.) and 12% of sub-dominant ( $< 1\%$  rel. Abund.) human faecal OTUs. A combination of the five highest-scored media (YCFA, GAM, BHI, GMM and Conditioned Medium) covered ca. 40% of human faecal OTUs.

The combination of SCD and gut content enrichments is promising and is being tested further for maximal recovery of the cultured diversity from human and mouse gut microbiota.

---

### 136-MEEV

Distribution patterns of *Brocadia* and *Nitrospiraceae* reflect high spatial heterogeneity of anammox and nitrification in oligotrophic groundwater

\*M. Krüger<sup>1</sup>, B. Thamdrup<sup>2</sup>, K. U. Totsche<sup>3</sup>, K. Küsel<sup>1,4</sup>, M. Herrmann<sup>4</sup>

<sup>1</sup>Friedrich Schiller University, Institute of Biodiversity, Jena, Germany

<sup>2</sup>University of Southern Denmark, Department of Biology, Odense, Denmark

<sup>3</sup>Friedrich Schiller University, Institute of Geoscience, Jena, Germany

<sup>4</sup>German Center for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Anthropogenic inputs of nitrate into groundwater pose a considerable risk to drinking water quality. However, groundwater chemistry is also affected by continued in situ microbial nitrate formation and nitrate reduction to gaseous nitrogen or ammonium. Here, we investigated nitrogen transformation processes and their microbial key players in oligotrophic karstic limestone aquifers in western Thuringia, Germany. These aquifers are characterized by a high spatial heterogeneity in the availability of oxygen, ammonium and nitrate. Under anoxic conditions, anaerobic ammonia oxidation (anammox) was the dominant N<sub>2</sub>-forming process at maximum rates of 5 nmol N<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup>, compared to almost undetectable denitrification activity. Variation of anammox activity and transcriptional activity of *hzsA* genes encoding

hydrazine synthase across sites pointed to the existence of anammox hotspots, where *Brocadia* accounted for up to 15% of the groundwater bacterial community. Abundances of archaeal and bacterial ammonia-oxidizers, including complete ammonia-oxidizers, were positively correlated with ammonium, while nitrite-oxidizing bacteria showed positive correlation with their product nitrate. Similar to anammox, transcriptional activity of *amoA* genes encoding ammonia monooxygenase and process rates suggested that nitrification activity was limited to a few oxic sites, where *Nitrospiraceae* formed up to 20% of the microbial community. Our results demonstrate a large spatial heterogeneity of aerobic and anaerobic nitrogen transformation processes in oligotrophic limestone aquifers. Availability of nitrogen compounds and oxygen are closely linked to the distribution patterns of the microbial key players but their activity, in turn, is likely to also shape groundwater nitrogen chemistry.

### 137-MEEV

#### A novel promiscuous *Roseobacter* plasmid mediates antibiotic resistance in the ocean

\*L. Birmes<sup>1</sup>, H. Freese<sup>1</sup>, J. Petersen<sup>1</sup>

<sup>1</sup>Leibniz-Institute DSMZ (German Collection of Microorganisms and Cell Cultures), Microbial Ecology and Diversity Research, Braunschweig, Germany

#### Background

Plasmids play an important role in the survival strategies of roseobacters (*Rhodobacteraceae*) in marine ecosystems. Their conjugation, which is driven by characteristic type IV secretion systems (T4SSs), mediates the horizontal transfer of important genes and is supposed to trigger the colonization of new environmental niches.

#### Methods

1. BLASTP: Identification of RepC\_soli-type plasmids
2. Comparative plasmid analyses: Discovery of crucial genes
3. Functionality test: Cloning of plasmid replicase & transformation in model organism (*Phaeobacter inhibens* DSM 17395)
4. Plasmid stability test: Spontaneous loss
5. Experimental conjugation: Intra- & inter-genus
6. Antibiotic resistance tests: Minimal inhibitory concentration; growth curves

#### Results

We discovered a novel *Rhodobacteraceae*-specific plasmid type designated RepC\_soli. As a unique selling point, the low copy number plasmid comprises a solitary replicase (*repC*) and lacks a typical *parAB* partitioning system. The conserved backbone of all RepC\_soli-type plasmids also contains solitary *parA* and *parB* genes, a T4SS and a toxin/anti-toxin module. Phylogenetic analyses of RepC\_soli proteins revealed the presence of four compatibility groups. We experimentally confirmed the functionality of the replicase and documented an exceptional stability of the 57 kb plasmid from *P. inhibens* P72 (pP72\_e). The natural chloramphenicol (Cm) resistance gene of pP72\_e served as selective marker for experimental conjugation across genus borders. Plasmid transfer caused a 20 to 40-fold increase of Cm resistance in the transconjugants.

#### Conclusions

The discovery of a *Rhodobacteraceae*-specific low copy number plasmid type without the archetypal *parAB* operon is indicative of a new mechanism of plasmid partitioning. The universal presence of T4SSs, experimental conjugation and the natural distribution of RepC\_soli plasmids reflect a highly promiscuous life style. The natural presence of plasmid-located antibiotic resistance genes in marine bacteria likely documents the anthropogenic footprint in the ocean.

#### Reference

Petersen, Vollmers et al. (2019) PNAS 116: 20568-20573, doi:10.1073/pnas.1905878116

### 138-MEEV

Tree root-associated microbiomes across Europe: respective impacts of sites' specificities and an oak clone

\*J. D. D. Habiaremye<sup>1,2,3</sup>, K. Goldmann<sup>1</sup>, T. Reitz<sup>1,4</sup>, S. Herrmann<sup>1,4</sup>, F. Buscot<sup>1,2,4</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Soil Ecology, Halle (Saale), Germany

<sup>2</sup>Leipzig University, Biology II, Leipzig, Germany

<sup>3</sup>University of Rwanda-College of Education, Mathematics, Science and Physical Education, Kigali, Rwanda

<sup>4</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Soil microbial communities are site specific and vary in response to changes of environmental factors. There are thus dissimilar microbial communities under different soil conditions or along natural gradients. In addition, tree roots recruit specific microbial partners from local soil communities and support their growth by exudates. Hence, the tree root-associated microbiome is shaped by both the host tree and environmental conditions. On this background, we analysed soil microbiomes in the root vs. root free zones of genetically identical host tree saplings, called PhytOakmeter (*Quercus robur* L., clone DF159), released at four grassland sites across a Europe North-South transect, from Finland to France. Soil microbial DNA was extracted, 16S and ITS2 genes were amplified to respectively target bacteria and fungi, and sequenced using Illumina MiSeq technology. Microbial communities were analysed in relation to soil chemistry and host tree growth. Species richness and Shannon diversity index in the tree root zone increased southwards for bacteria but were constant for fungi. Within southern sites of the transect, microbial diversity was higher in PhytOakmeter root zones than in the tree root-free zones while the middle and northern sites showed similar diversity values between the two soil compartments. Analysis of similarities permutations together with non-metric multidimensional scaling showed a site effect on the analysed microbial communities. Soil parameters like pH, TOC, TON, C/N and the clonal host tree growth impacted on microbial diversity and community composition. Very young PhytOakmeters exerted a remarkable influence on soil microbial diversity and community structure, and were able to select specific microbial genera, enriched in ectomycorrhizal fungi, yeasts and saprobes from respective local microbial pools. This validates the PhytOakmeter approach to better unravel recruitment processes of microbial partners by host trees at large spatial scales and under broad range of environmental conditions.

### 139-MEEV

The lag phase in batch cultivation is determined by differential growth rather than adaptation

\*P. Mishra<sup>1</sup>, R. U. Meckenstock<sup>1</sup>

**Introduction:** The lag phase is the early phase of microbial growth in batch cultures, when bacteria adapt to the conditions after being inoculated to new growth medium. According to textbooks, bacteria do not divide during this adaption period but rather increase in cell size, repair damages, and produce enzymes needed to metabolize the substrate.

**Objectives:** In contrast to the established concept, we hypothesize that the lag phase appears to be an adaptation phase because only a small fraction of the population starts growing exponentially immediately after inoculation whereas the majority of cells remain inactive. Since the number of growing cells is small compared to the inactive background, the total number of cells appears seemingly constant. As soon as the initially small number of exponentially dividing cells outnumbers the inactive cells, the populations increases to a measurable extent and the batch culture eventually enters the log-phase.

**Materials and methods:** To evaluate this hypothesis, we studied the growth of *Pseudomonas fluorescens* with <sup>13</sup>C-labelled glucose and deuterated water in the growth medium at the single cell level. Only actively growing cells incorporated both labels into their biomass which could be followed with Raman microspectroscopy

**Results:** Raman-microscopy revealed the incorporation of <sup>13</sup>C and deuterium into a small fraction of cells (7%) already 60 min after inoculation, indicating active metabolism. 300 min after inoculation, the total cell number and the optical density started to increase when the initially small number of labelled cells increased from 7% to over 90% of all cells. Interestingly, we also observed heterogeneity in metabolic traits in the cells of same population at different growth phases.

**Conclusion:** We conclude that the present concept of the lag phase is merely a phenomenological description and does not reflect a particularly cell physiology. Instead, the lag phase is determined by the ratio of active to inactive cells and the time which the actively dividing cells need to outnumber inactive cells.

---

#### 140-MEEV

Mechanistic microbial ecosystem model inference: A new method to analyze time series data

\*F. Hellweger<sup>1</sup>, J. Hoffmann<sup>1</sup>

<sup>1</sup>TU Berlin, Wasserreinhaltung, Berlin, Germany

Modern molecular observational technologies (e.g. sequencing, probes) can produce high-resolution time series of microbial concentrations. The dynamics in those data hold important clues about the ecology and interactions of the various system components (phytoplankton, DOM, bacteria). These datasets are typically analyzed using empirical methods (e.g. correlation networks), but those provide limited mechanistic insight (e.g. a network may show interactions between two viruses) and are of limited use for making predictions. Mechanistic approaches do not suffer from these limitations, but are generally not applied to big data sets, maybe because of the large number of processes and components.

Here we introduce mechanistic microbial ecosystem model inference (MMEI), where the model structure, including

components, processes and parameters are optimized within mechanistic constraints to match observed dynamics. The model allows for any number of components that interact via a several processes, including exudation, photosynthesis, grazing, heterotrophy and others. The optimization problem is challenging because of the many components, non-linear interactions and feedbacks, which is handled using a method that mimics natural speciation or diversification (e.g. stepwise increase in the number of phytoplankton species).

The MMEI method is applied to a four-year, daily resolution marine microbial time series, including observations for 20 phytoplankton and 40 bacteria types. The resulting mechanistic model includes additional 30 POM and DOM types. The following results are discussed: (a) Model calibration (years 1-3) and validation (year 4); (b) Predicted interactions, including specific phytoplankton > DOM > bacteria linkages, and comparison to auxiliary data (e.g. functional gene contents); (c) Predicted mass fluxes between ecological compartments and to the deep ocean; and (d) Functioning under a warmer future climate.

---

#### 141-MEEV

Analysis of microbial ecology by mass spectrometry-based metabolomics techniques

\*L. Castaño<sup>1</sup>, P. Rakowska<sup>2</sup>, B. Johnston<sup>2,3</sup>, M. Chrubasik<sup>3</sup>, K. Duncan<sup>1</sup>

<sup>1</sup>Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Microbiology, Glasgow, United Kingdom

<sup>2</sup>National Physical Laboratory, NiCE-MSI, Teddington, United Kingdom

<sup>3</sup>Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom

Mass spectrometry-based metabolomics has become a powerful tool for the study of chemical ecology. With the recent advances of mass spectrometry technologies and bioinformatics tools it is now possible to study both; all the microorganisms present in a specific environment at a specific time and the interactions with the environment and within microorganisms living in the same ecological niche. The bacterial order actinomycetales are responsible for the production of 65-70% of microbially produced specialised metabolites with diverse biological activities. Some actinomycetale strains contain over 30 biosynthetic gene clusters encoding for these metabolites. However, it is estimated that only 10% of these genes are transcribed in a mono-culture setting. Furthermore, it has been observed that microbial interactions may induce cryptic gene clusters providing a defence mechanism. Therefore, the study of bacterial competition it is key in understanding the ecological role that secondary metabolites pose to microbes and in understanding the induction of cryptic biosynthetic gene clusters as a result of these microbial interactions. Methods: microbial interactions were assessed in tri-cultures (three strains) and co-cultures (two strains) using 49 actinomycetale strains, two *Pseudomonas* and one *Bacillus* strain. Metabolites were extracted for interesting interactions and assessed with mass spectrometry-based metabolomics techniques such as liquid chromatography mass spectrometry (LC-MS), ToF-SIMS (imaging mass spectrometry) and MetaboAnalyst. Results: It was found that 29 strains showed altered phenotypes as a result of bacterial competition. The analysis of these interactions with LC-MS revealed the production of metabolites that were specific to the tri-cultures and co-cultures. Furthermore, these interactions, were subjected to imaging mass spectrometry in order to visualize the distribution of metabolites within the samples which allowed a better understanding of the chemical ecology during bacterial competition. Conclusions: mass spectrometry represents an exciting strategy to study bacterial chemistry and the ecology of bacterial competition.

---

## 142-MEEV

Negative hysteresis as a novel principle to improve antibiotic therapy

\*H. Schulenburg<sup>1</sup>, R. Roemhild<sup>1</sup>

<sup>1</sup>Max-Planck Institute for Evolutionary Biology, Plön, Germany

**Introduction:** Evolutionary processes are responsible for the current antibiotic crisis. Surprisingly, they are usually ignored during design of novel therapy. In general, bacteria show enormous potential to adapt to constant environments, including those defined by antibiotics. Adaptation may however be more difficult if conditions change fast. Thus, rapid fluctuations in drug application may prevent resistance development, especially if the used antibiotics sensitize bacterial cells towards subsequently administered antibiotics (i.e. the phenomenon of negative hysteresis). To date, fast fluctuations and negative hysteresis are not considered for antibiotic therapy.

**Objectives:** The aim of our study is to test experimentally whether fast switches between antibiotics and negative hysteresis minimize the ability of bacteria to adapt to drug therapy.

**Methods:** The model pathogen *Pseudomonas aeruginosa* was subjected to several evolution experiments with constant or fluctuating antibiotic treatments, followed by phenotypic analyses and whole genome sequencing combined with functional genetic analysis to identify possible targets of selection.

**Results:** Treatment with beta-lactam antibiotics induces long-lasting sensitization of *P. aeruginosa* towards aminoglycoside drugs. High levels of such negative hysteresis improve efficacy of fast sequential antibiotic therapy through three complementary effects: increased bacterial clearance, reduced adaptation rates, and reduced multidrug resistance. Genomic and functional genetic analysis revealed that negative hysteresis imposes specific selective constraints that favor an evolutionary response to the inducible sensitizing effect instead of the evolution of multidrug resistance.

**Conclusion:** Negative hysteresis increases bacterial extinction in fast sequential treatments and reduces selection of resistance by favoring responses specific to the induced physiological effects. Fast changes between antibiotics are key, because they create the continuously high selection conditions that are difficult to counter by bacteria. Our study highlights how an understanding of evolutionary processes can help to outsmart human pathogens.

---

## 143-MEEV

**Heatwaves cause long-term restructuring of freshwater bacterioplankton communities mediated by dispersal and zooplankton grazing**

\*M. Bengtsson<sup>1</sup>, D. Murray<sup>2</sup>, K. Mironova<sup>3</sup>, A. Abonyi<sup>4</sup>, A. Endredi<sup>5</sup>, C. Vad<sup>4</sup>, R. Ptacnik<sup>4</sup>

<sup>1</sup>University of Greifswald, Institute of Microbiology, Greifswald, Germany

<sup>2</sup>University of East Anglia, Norwich, United Kingdom

<sup>3</sup>Severtsov Institute of Ecology and Evolution, Moscow, Russian Federation

<sup>4</sup>WasserCluster Lunz, Lunz, Austria

<sup>5</sup>Hungarian Academy of Sciences, Budapest, Hungary

Heatwaves are expected to be more frequent and intense in the future and will cause significant disturbance to vulnerable

ecosystems. Alpine lakes may be especially impacted due to their cold-adapted communities and their restricted biodiversity. Bacteria perform essential ecosystem services in lakes, such as degrading pollutants, remineralizing carbon and acting as a food source for protists and zooplankton, thereby fueling aquatic foodwebs. The impact of heatwaves on bacteria in real-world ecosystems is likely complex as they are both directly and indirectly affected by temperature; via their own metabolism, via interactions with temperature-sensitive phytoplankton and via top-down control of their heat-stressed predators. We manipulated heat as well as dispersal of microbes and zooplankton in a mesocosm experiment featuring complex plankton communities from natural lakes. Mesocosms were monitored using both molecular and traditional plankton profiling in order to disentangle dynamics and interactions. We hypothesized that a week-long heatwave (+5 C) would lead to increases in bacterial activity during the heatwave and long-term changes in bacterial community composition depending on dispersal. Our results revealed increases in bacterial activity during the heatwave, but only in combination with dispersal. Strong shifts in community composition due to the heatwave lasted for over two weeks after the heatwave had ended. We found that zooplankton abundance explained more variation during the heatwave, while heat explained more variation after two weeks, indicating legacy effects of impaired top-down control during the heatwave. Potentially pathogenic bacteria such as *Legionella* sp. were overrepresented in heated tanks while several *Bacteroidetes* taxa were underrepresented. In conclusion, heatwaves alter freshwater bacterial communities on timescales that exceed the duration of the heatwave itself. Changes in top-down control is likely more important than direct effects of temperature in driving bacterial community composition, which implies that understanding plankton foodweb dynamics is key for predicting the microbial consequences of a warmer world.

---

## 144-DKMV

Evolutionary trajectories of antibiotic resistance in *Pseudomonas aeruginosa* generated in a morbidostat device

\*M. Javed<sup>1</sup>, B. Jentzsch<sup>1</sup>, V. Ueltzhoeffer<sup>1</sup>, M. Heinrich<sup>1</sup>, R. Neher<sup>2</sup>, M. Willmann<sup>1</sup>

<sup>1</sup>University of Tübingen, Tübingen, Germany

<sup>2</sup>University of Basel, Biozentrum, Basel, Switzerland

**Introduction**

Colistin is a last resort antibiotic against the critical status pathogen *Pseudomonas aeruginosa*.

**Objectives**

The aim of this study is to investigate the evolutionary trajectories towards antibiotic resistance using five clinical strains of *P. aeruginosa* which have been exposed to increasing quantities of colistin and metronidazole and a combination of those drugs over 21 days in a morbidostat device.

**Materials and Methods**

Five assays were performed to elucidate the effect of antibiotic resistance on the phenotype of each strain: growth curves, two biofilm assays, a serum killing assay, and a virulence assay using *Galleria mellonella*.

**Results**

The cell density in biofilm increases under exposure to colistin, while the addition of metronidazole can remove this effect. This indicates that metronidazole may modulate the effect of colistin on the development of vital cells in biofilms. We furthermore observed an overall increase in biomass within biofilms for both single-drug and combination..

The killing effect of serum is lost within seven days of colistin exposure. The control condition strains maintain their susceptibility to serum. This suggests that colistin drives bacterial modifications conferring a protective effect from serum complement factors.

Almost all strains show a decrease in virulence as the number of days to colistin exposure continues, in contrast to the control conditions.

## Conclusion

Significant changes are underway with regards to the phenotype of the strains after exposure to colistin and the combination of colistin and metronidazole. Our results suggest a clinically important bacterial evolution under sublethal antibiotic concentration.

---

## 145-DKMV

The author has not agreed to a publication.

---

## 146-DKMV

Antimicrobial efficiency of antisense PNAs in *Streptococcus pyogenes*

\*G. Barkowsky<sup>1</sup>, A. L. Lemster<sup>1</sup>, R. Pappesch<sup>1</sup>, S. Krüger<sup>1</sup>, A. Jacob<sup>2,3</sup>, B. Kreikemeyer<sup>1</sup>, N. Patenge<sup>1</sup>

<sup>1</sup>University Medicine Rostock, Institute of Medical Microbiology, Virology and Hygiene, Rostock, Germany

<sup>2</sup>Deutsches Krebsforschungszentrum, Functional Genome Analysis, Heidelberg, Germany

<sup>3</sup>Peps4LS, Heidelberg, Germany

## Introduction

*Streptococcus pyogenes* is an exclusively human pathogen causing a wide range of clinical manifestations from mild superficial infections to severe, invasive diseases. *S. pyogenes* is consistently susceptible toward penicillin, but therapeutic failure of penicillin treatment has been reported frequently. At the same time, streptococcal resistance to alternative antibiotics is common. Therefore, novel therapies are needed. Antisense peptide nucleic acids (PNAs) were shown to reduce growth of several pathogenic bacteria, but spontaneous cellular PNA uptake is restricted.

## Objectives

PNAs can be coupled to cell-penetrating peptides (CPPs) that support PNA translocation. Here, we investigate the effect of 18 CPP-coupled anti-*gyrA* PNAs on *S. pyogenes* growth and virulence.

## Materials and methods

*S. pyogenes* was incubated with CPP-coupled anti-*gyrA* PNAs for 6 h at 37 °C. Reduction of bacterial counts was determined in comparison to the untreated sample. CPP-PNA minimal inhibitory concentrations (MICs) were determined according to the protocol of Clinical and Laboratory Standards Institute ("CLSI"). The *gyrA* transcript

level following treatment was determined by RT-qPCR. Additionally, we tested the effect of CPP-anti-*gyrA* PNAs in a *Galleria mellonella* infection model.

## Results

HIV-1 TAT, oligolysine (K8), and (RXR)4XB peptide-coupled anti-*gyrA* PNAs efficiently abolished bacterial growth *in vitro*. The MIC of TAT- and K8-anti-*gyrA* PNAs was 15.6 µM and of (RXR)4XB-anti-*gyrA* 62.5 µM. The level of *gyrA* mRNA was reduced in bacteria challenged with these three CPP-anti-*gyrA* PNAs. Treatment with antimicrobial effective CPP-PNAs increased survival of larvae in the *G. mellonella* infection model.

## Conclusion

Out of 18 CPPs, we could identify three CPPs (TAT, K8, and (RXR)4XB) which support the uptake of PNAs in *S. pyogenes*. For treatment of *S. pyogenes* infections, these CPPs could be coupled to other essential genes or to antibiotic resistance genes.

---

## 147-DKMV

Therapeutic drug monitoring of antibiotics in intensive care patients treated with extracorporeal membrane oxygenation (ECMO): an observational single-center study

\*D. Kühn<sup>1</sup>, C. Metz<sup>1</sup>, H. Wehrfritz<sup>1</sup>, M. Alqudrah<sup>1</sup>, A. Becker<sup>1</sup>, M. Hoffmann<sup>1</sup>, R. Bals<sup>1</sup>, U. Hübner<sup>1</sup>, J. Geisel<sup>1</sup>, P. Lepper<sup>1</sup>, \*S. Becker<sup>1</sup>

<sup>1</sup>Saarland University, Homburg/Saar, Germany

**Introduction:** Effective antimicrobial treatment is key to reduce the high mortality of bacterial sepsis in patients on intensive care units (ICUs). Dose adjustments are often necessary to account for pathophysiological changes or renal replacement therapy. Extracorporeal membrane oxygenation (ECMO) is increasingly being used for the treatment of respiratory and/or cardiac failure. However, it remains unclear whether dose adjustments are necessary to avoid subtherapeutic drug levels in septic patients on ECMO support.

**Objective:** To evaluate and comparatively assess the mean serum concentrations of continuously applied antibiotics in intensive care patients being treated with and without ECMO.

**Materials and methods:** Between November 2018 and October 2019, we prospectively enrolled patients on a pneumological intensive care unit in southwest Germany who received antibiotic treatment with piperacillin/tazobactam, ceftazidime, meropenem, or linezolid. All antibiotics were applied using continuous infusion, and therapeutic drug monitoring of serum concentrations (expressed as mg/L) was carried out using high-performance liquid chromatography. Target concentrations were defined as 4-fold above the minimal inhibitory concentration (MIC) of susceptible bacterial isolates, according to EUCAST breakpoints.

**Results:** The final cohort comprised 86 ICU patients, of whom 24 were treated with ECMO. ECMO patients were significantly younger (mean age: 47.9 vs. 62.2 years), required haemodialysis more frequently (58.3% vs. 32.3%) and had an elevated ICU mortality (54% vs. 23%). In the ECMO group, mean antibiotic serum concentrations were significantly lower for piperacillin (43.6 vs. 78.1), high-dose meropenem (24.2 vs. 42.8) and linezolid (8.5 vs. 14.8). We found high rates of insufficient antibiotic serum concentrations among ECMO patients, which did not reach

the pre-specified MIC target (piperacillin: 55% vs. 14% in non-ECMO; linezolid: 37% vs. 8%). No such difference was observed for ceftazidime and meropenem.

**Conclusions:** ECMO treatment was associated with significantly reduced serum concentrations of piperacillin and linezolid.

---

#### 148-DKMV

Review on 5 years of screening for carbapenemase-producing strains

\*N. Dietze<sup>1</sup>, N. Lippmann<sup>1</sup>, A. C. Rodloff<sup>1</sup>

<sup>1</sup>Institut für Medizinische Mikrobiologie, Diagnostik, Leipzig, Germany

#### Introduction

The spread of multiresistant strains is an increasing threat for hospitalized patients especially if carbapenemase producing strains (CPS) are the cause of an infection. Extensive data on asymptomatic CPS carriers are still missing. After an outbreak of carbapenemase producing *Klebsiella pneumoniae* at the University hospital of Leipzig (2010-2013), the hospital was constantly looking for ways to avoid transmission between patients. Beside a strict adherence to hygienic orders, the clinic developed a consistent screening system.

#### Objectives

The following study is the first one to describe the outcome of a CPS screening after a carbapenemase outbreak.

#### Materials & methods

Stool specimens/rectal swabs were screened for CPS by cultural and molecular biological methods. Patient data were analyzed using the medical records.

#### Results

In total 92768 patients (2012-2017) were screened for the presence of CPS. Amongst them 220 (0.24%) patients were found to be carrier of CPS, out of these 240 different CPS strains were isolated. In detail 39.6% (n=95) VIM, 22.9% (n=55) OXA-48, 22.5% (n=54) KPC, 11.3 % (n=27) NDM-1, 1.3% (n=3) IMP and 0.8% (n=2) GES carbapenemases were detected. Four strains were found to be positive for two different carbapenemases: VIM/OXA-48 (n=1), VIM/IMP (n=1), VIM/GES (n=1), VIM/NDM-1 (n=1). In 68.2% (n=150) cases the CPS was detected at the admission to the hospital, 32.8% were detected due to other screening reasons: 20.9% (n=46) patients hospitalized for more than 14 days, 10.5% (n=23) CPS in other material than stool/rectal swab, 0.5% (n=1) patient with contact to a CPS patient. In 16 patients (7.3%) the CPS was isolated from blood cultures indicating an endogenous infection.

#### Conclusions

Besides the presenting of the screening results, the study should lead to the discussion, if an extensive screening of hospitalized patients has any positive effects for patients and hospitals. However, more detailed knowledge about the duration of colonization and possible risk factors for a CPS carriage might be useful to prevent endogenous infections with resistant gram negative bacteria.

---

#### 149-DKMV

The author has not agreed to a publication.

---

#### 150-DKMV

Gram-negative bloodstream infections: a quality of care assessment

\*C. L. Correa-Martinez<sup>1</sup>, H. Tönnies<sup>1</sup>, A. Mellmann<sup>1</sup>, S. Kampmeier<sup>1</sup>

<sup>1</sup>University Hospital Münster, Institute of Hygiene, Münster, Germany

#### INTRODUCTION

Bloodstream infections caused by Gram-negative bacteria (GNB) are a cause of sepsis, which remains the most important cause of death among infections in Germany. The adequate management of GNB bacteremia (GNBB) can be hampered by the lack of specialized assessment and optimal communication between clinicians and microbiology experts. In contrast to *Staphylococcus aureus* bacteremia, treatment of GNBB is difficult to standardize due to the variety of causal agents and resistance mechanisms involved.

#### OBJECTIVES

The aim of this study was to evaluate the quality of the GNBB management at the University Hospital Münster (UHM).

#### MATERIALS AND METHODS

Five quality-of-care indicators (QCI) were defined to evaluate the management of GNBB cases: 1) antibiotic adjustment according to microbiological results, 2) dosage, 3) follow-up blood cultures, 4) duration of treatment and 5) source identification. One point was assigned to each QCI correctly addressed (total score: 0-5). GCI scores were calculated for all GNBB cases detected over a 6-month period at the UHM.

#### RESULTS

In total, 79 GNBB cases were identified. The patients' average age was 59 years and 69.6% of them were male. In relation to the total number of cases, QCI were satisfactorily covered as follows: 1) 48.1%; 2) 34.2%; 3) 63.3%; 4) 57.0%; 5) 59.5%. The average QCI score was 3. The most commonly isolated bacteria were *Escherichia coli* (67.1%) and *Klebsiella* spp. (13.9%). GNBB was defined as hospital-acquired (diagnosis >48 h after admission) in 47 cases (59.5%).

#### CONCLUSION

GNBB is a potentially lethal condition that poses a challenge for the clinician. At our hospital, therapeutic management of GNB requires optimization. Measures should be taken aiming at an overall quality improvement with a special focus on antibiotic selection and dosage. On-site ABS consultations have been shown to positively impact clinical practices and could thus contribute to increase the quality of GNBB management.

---

#### 151-DKMV

Antimicrobial resistance of bacteraemia in the emergency department of a German university hospital (2013–2018): potential carbapenem-sparing empiric treatment options in light of the new EUCAST recommendations



\*K. Rothe<sup>1</sup>, N. Wantia<sup>1</sup>, D. Busch<sup>1</sup>, B. Waschulzik<sup>2</sup>, C. Spinner<sup>3</sup>

<sup>1</sup>Technical University of Munich, Institute for Medical Microbiology, Immunology and Hygiene, Munich, Germany

<sup>2</sup>Technical University of Munich, Institute of Medical Informatics, Statistics, and Epidemiology, Munich, Germany

<sup>3</sup>Technical University of Munich, University Hospital rechts der Isar, Department of Medicine II, Munich, Germany

**QUESTION:** This study investigated predominant microorganisms causing community-onset bacteraemia at the medical emergency department (ED) of a tertiary-care university hospital in Germany from 2013 to 2018 and their antimicrobial susceptibility patterns.

**METHODS:** Antimicrobial resistance patterns in patients with positive blood cultures presenting to an internal medicine ED were retrospectively analysed.

**RESULTS:** Blood cultures were obtained at 5,191 of 66,879 ED encounters, with 1,013 (19.5%) positive results, and true positive results at 740 encounters (diagnostic yield, 14.3%). The most frequently isolated relevant microorganisms were *Enterobacterales* (n=439, 59.3%), *Staphylococcus aureus* (n=92, 12.4%), *Streptococcus pneumoniae* (n=34, 4.6%), *Pseudomonas aeruginosa* (n=32, 4.3%), *Streptococcus pyogenes* (n=16, 2.2%), *Enterococcus faecalis* (n=18, 2.4%), and *Enterococcus faecium* (n=12, 1.6%). Antimicrobial susceptibility testing revealed a high proportion of resistance against ampicillin-sulbactam in *Enterobacterales* (42.2%). The rate of methicillin-resistant *Staphylococcus aureus* was low (0.4%).

Piperacillin-tazobactam therapy provided coverage for 83.2% of all relevant pathogens using conventional breakpoints. Application of the new European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations increased the percentage of susceptible isolates to high-dose piperacillin-tazobactam to 92.8% ( $p < 0.001$ ). Broad-spectrum carbapenems would only cover an additional 4.8%. The addition of vancomycin or linezolid extended coverage by just 1.7%.

**CONCLUSIONS:** Using an ureidopenicillin-beta-lactamase inhibitor combination at the high dose suggested by the new EUCAST recommendations provided nearly 93% coverage for relevant pathogens in patients with suspected bloodstream infection in our cohort. This might offer a safe option to reduce the empiric use of carbapenems. Our data support the absence of a general need for glycopeptides or oxazolidinones in empiric treatment.

## 152-PRV

Low energy electron irradiation is a promising novel method for sterilization of bacteria- or virus-containing liquids

\*J. Fertey<sup>1</sup>, S. Reißhauer<sup>1</sup>, B. Standfest<sup>2</sup>, M. Thoma<sup>2</sup>, J. Beckmann<sup>3</sup>, J. Portillo<sup>3</sup>, F. H. Rögner<sup>3</sup>, T. Grunwald<sup>1</sup>, S. Ulbert<sup>1</sup>

<sup>1</sup>Fraunhofer Institute for Cell Therapy and Immunology, Immunology, Leipzig, Germany

<sup>2</sup>Fraunhofer Institute for Manufacturing Engineering and Automation, Laboratory Automation and Biomanufacturing Engineering, Stuttgart, Germany

<sup>3</sup>Fraunhofer-Institute for Organic Electronics, Electron Beam and Plasma Technology, Dresden, Germany

### 1. Introduction

Irradiation with ionizing radiation is an effective sterilization method for pathogen containing solutions. However, high energy is needed for sterilizing liquids in standard processes, hence existing irradiation techniques (gamma-, X-rays and high-energy electron beam) require big and complex

radiation protection shielding constructions and thus cannot be used in a normal laboratory environment. We have previously demonstrated that low-energy electron irradiation (LEEI) is an efficient inactivation method for pathogens in liquids that does not require strong shielding and can therefore be integrated in any laboratory setting. In addition, it is extremely fast due to a high dose rate and optimally conserves biological structures, which is essential for many downstream-applications.

### 2. Objectives

We constructed a research-scale prototype for LEEI treatment of pathogen-containing liquids (milliliters up to several liters), that can be implemented in sterilization or manufacturing processes under GMP conditions.

### 3. Materials and Methods

We tested different bacteria (*E.coli* and *B. cereus*) and viruses (*InfluenzaA* and *Zika*) with LEEI using two different completely closed concepts for automation (a continuous inactivation system and a system based on disposable bags). The pathogens were tested for infectivity before and after LEEI treatment. Protein conservation was analyzed by ELISA using polyclonal antibodies. DNA of irradiated and non-irradiated samples was extracted and analyzed by PCR.

### 4. Results

A dose-dependent reduction of infectivity was observed, resulting in complete inactivation of all tested pathogens. As expected, bacteria required lower doses than spores or viruses. Compared to non-irradiated material, the antigenic structures in inactivated samples remained largely intact. Although LEEI treatment leads to fragmentation of nucleic acids, PCR detection (amplicons > 300bp) yielded positive results.

### 5. Conclusion

The unique prototype enables the efficient sterilization of pathogen containing liquids in a fast, automated process and allows the direct use of the material in sensitive diagnostic downstream assays such as ELISA or PCR.

## 153-PRV

Prevalence of multidrug resistant gram negative bacteria in siphons of ICU patient rooms with and without disinfection procedures

\*C. E. Belmar Campos<sup>1</sup>, G. Franke<sup>1</sup>, E. M. Klupp<sup>1</sup>, A. Kollotzek<sup>2</sup>, J. K. Knobloch<sup>1</sup>

<sup>1</sup>University Medical Centre Hamburg-Eppendorf, Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany

<sup>2</sup>University Medical Centre Hamburg-Eppendorf, Hamburg, Germany

**Introduction** Sink siphons in hospitals, especially in the ICU, are considered to be potential sources of nosocomial infections and/or colonizations with multidrug resistant (MDR) pathogens. After experiencing an outbreak with MDR *P.aeruginosa* in 2015, disinfection of siphons in all ICU patient rooms with Perform ® 2% (Schülke, Hamburg, Germany) once a week and screening of these siphons for MDR bacteria were carried out. Disinfection procedures were adjusted in two wards in April 2017. In this study we analyzed samples of six patient rooms of these wards, for the period of 22 weeks before and after stopping disinfection procedure to check if there was an increase of MDR Gram

negative bacteria, especially such producing carbapenemases.

**Methods** In two ICU wards (one interdisciplinary, one surgical) siphons from the washbasin in six patient rooms were sampled weekly and analyzed for the presence of carbapenem resistant Gram negative organisms (CRGNO). Species ID was performed by mass spectrometry and AST using an automated system. Isolated CRGNO were screened by PCR for the presence of carbapenemase genes (KPC, NDM, Oxa-48 group and VIM).

**Results** No significant difference ( $p=0,4$ ) between the disinfection (19/132 CRGNO positive samples [14%]) and none disinfection period (25/132 CRGNO positive samples [19%]) was observed. The most frequent species detected was *P. aeruginosa* (56%). The other isolates belonged to the group of Enterobacterales (*K. pneumoniae* 19%, *E. cloacae* complex 17% and *C. freundii* 8%).

A carbapenemase gene was found in 84% of all tested isolates during the whole period with VIM (55%) and Oxa48 (45%) being the only genes detected.

**Conclusions** CRGNO are frequently observed in sink siphons in patient rooms in the ICU ward of our hospital. A high percentage of these isolates harbor a carbapenemase gene. Disinfection of siphons with Perform® could not prevent colonization with CRGNO and there was no relevant increase of such isolates after stopping disinfection procedures. Therefore, alternative prevention strategies to avoid nosocomial transmission of CRGNO via siphons in hospitals are required.

#### 154-PRV

Plasma activated water inactivates efficiently gram-negative and gram-positive microorganisms

\*M. Hummert<sup>1</sup>, P. H. M. Leenders<sup>2</sup>, P. van Ooij<sup>2</sup>, T. Kuczius<sup>1</sup>

<sup>1</sup>Institute of Hygiene, University of Münster, University Hospital of Münster, Münster, Germany

<sup>2</sup>VitalFluid B.V., Nijmegen, Netherlands

Water activated by atmospheric pressure plasma (PAW) has been proposed as a new approach in bacterial inactivation. During water activation many active and reactive substances and radicals arise associated with a radical decrease in pH. In this study we investigated the influence of PAW on antimicrobial properties tested with bacterial strains such as *Pseudomonas aeruginosa* as waterborne pathogen, *Escherichia coli* as fecal indicator and *Staphylococcus aureus* as skin colonizer.

Drinking water was activated by non-thermal atmospheric pressure plasma with electric power of 60 to 120 W for 10 to 30 min. In the following PAW was used for post-discharge-treatment of planktonic cells grown in nutrients and adapted to tap water for long term survival.

Strains were exposed to PAW for defined periods of time (2 to 120 min) followed by cultivation. After treatment PAW activity was stopped by application of neutralization solution. The PAW effect on bacteria was proved by live/dead differentiation using real-time polymerase chain reaction (PCR) with propidium monoazid (PMA) pre-treatment and flow-cytometry.

Post-discharge treatment was highly efficient against bacteria and the disinfecting properties of PAW were dependent on the activation conditions and the treatment

time for the water. Starting with 108 bacteria per mL, PAW (activated for 10 min at 100 W) achieved at least an 8-decimal-logarithm-unit reduction in 10 min treatment for all tested strains when grown in nutrient media. The treatments were less efficient on *P. aeruginosa* and *E. coli* when they were adapted to tap water and incubated for several weeks. PAW had to be activated with higher electric power (120 W) and prolonged activation (30 min) to achieve a reduction of at least 8 decimal-logarithm-units. These antimicrobial properties were upheld during storage of the PAW at room temperature for at least 120 min. PAW demonstrates a high potential in applications as disinfectant.

---

#### 155-PRV

**Removal of antibiotic residues, antibiotic-resistant bacteria and antibiotic-resistance genes from hospital wastewater, by using ultrasonic treatment**

\*C. Felder<sup>1</sup>, A. M. Voigt<sup>1</sup>, E. Sib<sup>1</sup>, F. Lenz-Plet<sup>1</sup>, D. Skutlarek<sup>1</sup>, H. Färber<sup>1</sup>, R. Schmithausen<sup>1</sup>, M. Exner<sup>1</sup>

<sup>1</sup>Universitätsklinikum Bonn, Institut für Hygiene und Öffentliche Gesundheit, Bonn, Germany

#### Introduction

Hospital wastewater is a hotspot for antibiotic residues, antibiotic-resistant bacteria and antibiotic-resistance genes. Especially with regard to reserve antibiotics, hospital wastewater is an important source for the spread of antibiotic resistances in the environment. In particular because conventional wastewater treatment plants are not able to fully remove these pollutants from the wastewater (Voigt et al. 2019).

#### Objectives

The aim of the study was to answer the question, whether it would be possible to reduce the concentrations of antibiotic residues, antibiotic-resistant bacteria and resistance genes in wastewater significantly, by using ultrasound treatment.

#### "Materials & methods"

For the simulation of the wastewater treatment, we used an ultrasonic reactor of the AIMES GmbH and a simulated Waste Water Matrix, purchased from Sigma-Aldrich (Taufkirchen, Germany). Concentrations of the antibiotic residues were measured with LC-MS/MS. Cultural analysis was performed by plating out a dilution series of the treated simulated wastewater onto chromogenic selective agar plates. The bacteria were differentiated via MALDI TOF MS and microdilution.

#### Results

With the help of ultrasound treatment, the concentration of antibiotic residues in wastewater can be reduced by up to 80 % within ten minutes. The amount of antibiotic-resistant bacteria and resistance also showed a great reduction.

#### Conclusion

Within the scope of the investigations, the principle of wastewater treatment by ultrasound has been applied for the first time to the removal of antibiotic residues, antibiotic-resistant bacteria and antibiotic-resistance genes. The study is a first step towards a decentralised in-line treatment of hospital wastewater to reduce the introduction of antibiotic resistance into the environment via hospital wastewater.

## Literature

Voigt, A. M.; Zacharias, N.; Timm, C.; Wasser, F.; Sib, E.; Skutlarek, D. et al. (2019): Association between antibiotic residues, antibiotic resistant bacteria and antibiotic resistance genes in anthropogenic wastewater - An evaluation of clinical influences. In *Chemosphere* 241, p. 125032. DOI: 10.1016/j.chemosphere.2019.125032.

---

### 156-PRV

Change of adherence to infection prevention measures in operating rooms as part of the HYGARZT Project

\*J. Hoffmann<sup>1</sup>, S. Herbrandt<sup>2</sup>, M. Neuwirth<sup>1</sup>, S. Akca<sup>3</sup>, U. Ligges<sup>2</sup>, C. Kugler<sup>3</sup>, F. Mattner<sup>1</sup>

<sup>1</sup>Kliniken der Stadt Köln, Institut für Hygiene, Köln, Germany

<sup>2</sup>Technische Universität, Fakultät Statistik, Dortmund, Germany

<sup>3</sup>Albert-Ludwigs-Universität Freiburg, Institut für Pflegewissenschaft, Freiburg i. Br., Germany

**Introduction** The BMG funded *HYGARZT* Project investigates medical and economic advantages of infection prevention and control measures (IPCM), implemented by the infection prevention and control link practitioner (IPC-LP) in orthopedics and trauma surgery.

**Objectives** Behavior of operation room (OR) staff, before and after the implementation of IPCM by the IPC-LP using a pre-after interventional study design.

**Methods and Materials** During the pre-intervention phase, adherence with existing IPC-measures was observed. We focused on hand hygiene (HH), wearing of caps and masks (CM), private conversation, opening of OR doors and fluctuation of OR staff. As intervention the OR staff was trained by the IPC-LP about the IPCMs, which were observed, by using a checklist. For statistical analysis we used the Fisher-Pitman permutation test.

**Results** Pre-/post-interventional 95/104 surgical procedures were observed, respectively. Overall 1945/2710 HH indications were identified. The adherence to HH before incision (excluding surgical hand disinfection) increased from 5.2% to 54.9% ( $p < 0.001$ ) (surgeons) and from 9.7% to 62.4% ( $p < 0.001$ ) (nurses), after surgery from 15.1% to 53.0% ( $p < 0.001$ ) (surgeons) and from 15.4% to 61.3% ( $p < 0.001$ ) (nurses). The adherence for correct donning of CM did not change significantly (wearing of surgical caps, about 60% ( $p = 0.390$ ) or surgical masks from 85.9% to 90.2% ( $p = 0.068$ )). Private conversations were documented in 19.2%/ 29.3% ( $p = 0.037$ ) of observed procedures. Door openings increased from 60.0% to 98.1% ( $p < 0.001$ ). In 31.6%/75.0% ( $p < 0.001$ ) of the procedures, persons entranced or leaved the OR.

**Conclusion** In our setting the IPC-LP accomplished significant risings in adherence to HH adherence for non-aseptic indications, carried out by the OR staff. Helpful was the definition correct moments for HH and simplified providing of disinfectants. In contrast, fluctuation of staff with associated door openings increased, which could be explained in part by personal exchange and shortages.

---

### 157-PRV

"When push comes to nudge": Is it time to nudge healthcare workers' compliance to prevent nosocomial infections and antimicrobial resistance? Results of two systematized

reviews on effects of nudging on hand hygiene and antibiotic use

J. Schewe<sup>1</sup>, \*T. von Lengerke<sup>1</sup>

<sup>1</sup>Hannover Medical School, Centre for Public Health and Healthcare, Department of Medical Psychology, Hannover, Germany

**Question** Healthcare workers' compliance to prevent nosocomial infections (NI) and antimicrobial resistance (AMR) remains suboptimal. To improve implementation, nudging has been introduced in the field.[1,2] Nudges refer to "any aspect of the choice architecture that alters people's behavior in a predictable way without forbidding any options or significantly changing...economic incentives", and choice architecture to influencing choice by "organizing the context in which people make decisions".[3] This study presents reviews on nudges aimed at hand hygiene compliance (HHC) and rational antibiotic use (RAU).

**Methods** Searches were conducted in PubMed and LIVIVO in April 2019 following PRISMA. Nudges in the studies were classified using appropriate typologies [4-5].

**Results** N=18 and N=13 studies using 26 and 19 nudges were included for HHC and RAU, respectively. Prompts, i.e. non-personalized information to raise awareness, were used most (24.4%). For HHC, these were followed by reminders (15.4%), frames (e.g. presentations of gains or losses), and proximal stimuli (e.g. increase of behavioral ease by placement; 11.5% each). For RAU, simplification, i.e. increasing ease of optimal choice, shared first place with prompts (21.1%), followed by frames, social norms, and availability (e.g. added behavioral options; 15.8% each). Effectiveness was reported for 71.1% of nudges. Of these, 77.7% (HHC) and 92.8% (RAU) reported statistically significant improvements.[TVL1]

**Conclusions** Nudging may represent a low-threshold approach to promote NI- and AMR- preventive compliance. The magnitude and ancillary conditions (e.g. motivation, regulations) of its effectiveness have yet to be determined.

[1] Jacob JT et al. Preventing healthcare-associated infections through human factors engineering. *Curr Opin Infect Dis* 2018;31:353-8

[2] Katchanov J et al. "Nudging" in microbiological reports. *Infection* 2017;45:123-7

[3] Hertwig R, Grüne-Yanoff T. Nudging and boosting. *Perspect Psychol Sci* 2017;12:973-86

[4] Hollands GJ et al. Altering micro-environments to change population health behaviour. *BMC Public Health* 2013;13:1218

[5] Sunstein CR. Nudging. *J Consum Policy* 2014;37:583-8

---

### 158-MPV

Caspase-3 dampens macrophage infiltration into *Staphylococcus aureus* abscess lesions

\*V. Winstel<sup>1,2,3</sup>, O. Schneewind<sup>3</sup>, D. Missiakas<sup>3</sup>

<sup>1</sup>TWINCORE, Centre for Experimental and Clinical Infection Research, Hannover, Germany

<sup>2</sup>Institute of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany

<sup>3</sup>University of Chicago, Department of Microbiology, Chicago, United States

*Staphylococcus aureus* is a notorious bacterial pathogen that causes fatal diseases in humans. During infection, *S. aureus* generates cytotoxic deoxyadenosine (dAdo) from neutrophil extracellular traps which eliminates phagocytes. Here, we demonstrate that disruption of caspase-3, a key modulator of apoptosis, protects macrophages from dAdo intoxication and impairs *S. aureus* disease pathogenesis. Mice lacking caspase-3 in immune cells exhibit diminished susceptibility towards *S. aureus* bloodstream infection, as caspase-3-deficient macrophages readily infiltrate abscess lesions thereby supporting neutrophils in the phagocytic clearance of staphylococci. Combined with a screen for single nucleotide polymorphisms (SNPs) in human *CASP3* that protect macrophages from *S. aureus*-mediated dAdo intoxication, these data may explain variable outcomes of *S. aureus* infections in humans.

## 159-MPV

### ***Staphylococcus epidermidis* biofilms alter the pro-inflammatory immune response in primary human macrophages**

\*S. Weißelberg<sup>1</sup>, A. Both<sup>1</sup>, H. Büttner<sup>1</sup>, H. Rohde<sup>1</sup>

<sup>1</sup>Universitätsklinikum Hamburg-Eppendorf, Medizinische Mikrobiologie, Virologie und Hygiene, Hamburg, Germany

## Question

Polarized macrophages are the first defense line of the human immune system. Since *S. epidermidis* evolved sophisticated mechanisms to escape the host's immune response professional phagocytes are facing problems eradicating those pathogens. In order to understand why and how biofilms are able to persist during this study investigated the cellular effects of macrophages during infection.

## Methods

Primary human macrophages isolated from buffy coats were infected with different *S. epidermidis* strains. Phagocytosis rates, macrophage polarization and TLR2 presentation were analyzed by confocal laser scanning microscopy and FACS analysis. For better understanding of the phagocyte's reaction during infection transcription and protein levels of IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and IL-10 were assessed and a RNAseq experiment was carried out.

## Results

Instead of being activated upon infection with bacteria embedded in a biofilm the macrophages' expression and secretion of pro-inflammatory cytokines is significantly reduced in contrast to contact with single cell bacteria. However, anti-inflammatory cytokines are highly upregulated. Along with these findings, the phagocytosis rate is dramatically decreased as soon as bacteria are protected in a biofilm. Interestingly, in an infection context the macrophages subtype is shifted towards anti-inflammatory M2 as confirmed in FACS analysis. The *S. epidermidis* recognition receptor is TLR-2. By blockage of this receptor the phagocytosis rate of biofilm positive strains can be recovered as well as the induction of the pro-inflammatory defense. When analyzing the overall gene expression pattern of macrophages during infection experiments it becomes clear that a dramatic dysregulation of the innate immune response takes place.

## Conclusion

With the help of biofilm formation bacteria are able to evade phagocytosis by shifting the host's immune response towards an anti-inflammatory profile. Understanding biofilm related immune escape mechanisms of *S. epidermidis* and other biofilm forming bacteria may pave the way towards novel therapeutic approaches in the future.

## 160-MPV

### **Coinfection of a porcine precision-cut lung slice model with *Bordetella bronchiseptica* and *Streptococcus suis***

\*D. Vötsch<sup>1</sup>, M. Willenborg<sup>1</sup>, W. Baumgärtner<sup>2</sup>, P. Valentin-Weigand<sup>1</sup>

<sup>1</sup>University for Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany

<sup>2</sup>University of Veterinary Medicine Hannover, Institute for Pathology, Hannover, Germany

*Bordetella (B.) bronchiseptica*, as well as *Streptococcus (S.) suis*, are respiratory pathogens contributing to the porcine respiratory disease complex (PRDC) which causes high economic losses in the pig industry. Furthermore, both are zoonotic pathogens and infect humans.

*S. suis* is a frequent colonizer of the upper respiratory tract of pigs, but it can also cause invasive infections in animals predisposed, e.g., by infection with *B. bronchiseptica*. Though the mechanisms how *B. bronchiseptica* promotes infection with *S. suis* are still unclear, it is plausible that *B. bronchiseptica* induced damage of the ciliated epithelium barrier facilitates adherence and invasion of *S. suis*.

In this study, we established a porcine precision-cut lung slice (PCLS) model to analyze interactions during coinfections with both pathogens. We investigated the effects of *B. bronchiseptica* infection on colonization, invasiveness and cell damaging capacities of *S. suis*, with particular focus on the role of its pore-forming toxin sulysin (SLY). For this purpose, we infected PCLS with a clinical isolate of *B. bronchiseptica* for 24 hours to predispose the PCLS to infection with a virulent *S. suis* serotype 2 strain and its isogenic SLY-deficient mutant strain, as well as the complemented mutant strain. During incubation, we determined the ciliary activity using light microscopy, the growth of *B. bronchiseptica* and *S. suis* in the supernatant by serial dilution and plating on agar plates and the extent of induced cell damage using an LDH release assay. Additionally, at certain time points, PCLS were lysed to evaluate cell-associated bacteria or were fixed for histopathology and immunofluorescence staining.

Taken together, our results showed that coinfection with *B. bronchiseptica* promoted adherence and cell damaging effects of *S. suis* independent of the presence of SLY. In addition, the PCLS coinfection model may also be used to study bacteria-bacteria-host interactions *ex vivo* in other porcine respiratory infections, as well as in human infections, considering that pigs are generally recognized as a suitable model for human diseases.

## 161-MPV

### **Zinc metalloprotease ProA contributes to dissemination of *Legionella pneumophila* in human lung tissue explants**

\*L. Scheithauer<sup>1</sup>, S. Thiem<sup>1</sup>, S. Schmelz<sup>2</sup>, A. Dellmann<sup>3</sup>, K. Büssow<sup>2</sup>, R. M. H. J. Brouwer<sup>4</sup>, C. Ünai<sup>1</sup>, W. Blankenfeldt<sup>2</sup>, M. Steinert<sup>1,5</sup>

<sup>1</sup>Technische Universität Braunschweig, institute of microbiology, Braunschweig, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, structure and function of proteins, Braunschweig, Germany

<sup>3</sup>Klinikum Braunschweig, pathology, Braunschweig, Germany

Legionnaires' disease is a severe form of pneumonia that can be life-threatening. One out of ten patients dies in consequence of an infection with the causative agent *Legionella pneumophila*. The bacterium is well-adapted to colonize man-made water systems and, after inhalation, to replicate in alveolar macrophages. Using human explants of pulmonary tissue we characterized the thermolysin-like protease ProA as a *L. pneumophila* virulence factor. Vital lung specimens were derived from cancer patients undergoing lobe- or pneumectomy, and histological events were investigated after inoculation with the purified protease. ProA treatment results in effective degradation of collagen IV, an important structural host factor. This evoked tissue destruction in the human lung samples, which facilitates dissemination of the pathogen and was similarly observed in infected patients. Purifying high amounts of recombinant ProA we additionally crystallized the protease and managed to determine the X-ray structure at 1.5 Å resolution. ProA consists of two domains with N-terminal  $\beta$ -sheets and C-terminal  $\alpha$ -helices, and contains a central zinc cofactor in the active site. Moreover, the crystal structure revealed vast homology to important virulence factors of the same enzyme family, such as pseudolysin from *Pseudomonas aeruginosa*. In molecular detail we were able to gain comprehension concerning the interplay of structure and function of ProA in human lung tissue explants.

## 162-MPV

### The role of the global regulators *sarA* and *sae* in *S. aureus* biofilm formation *in vitro* and *in vivo*

H. Van de Vyver<sup>1</sup>, M. Fröhlich<sup>1</sup>, S. Schelhaas<sup>2</sup>, S. Hermann<sup>2</sup>, M. Herrmann<sup>1</sup>, M. Kuhlmann<sup>2</sup>, \*S. Niemann<sup>1</sup>

<sup>1</sup>University Hospital Münster, Institute of Medical Microbiology, Münster, Germany

<sup>2</sup>Münster University, European Institute for Molecular Imaging, Münster, Germany

*Staphylococcus aureus* causes very severe infections of vascular grafts. To study the pathogenesis of these infections we have developed a murine model that closely mimics the situation as it is in patients by implanting a Teflon catheter inside the carotid artery of mice followed by intravenous infection with *S. aureus*. In this model the biofilm is formed under the stress of high blood flow as well as the pressure of the host immune system. In this study we investigated the role of the global regulators *sarA* and *sae* in biofilm formation *in vitro* and *in vivo*.

**Methods:** A catheter was placed in the right carotid artery of CD1-mice. Mice were infected with *S. aureus* LS1 wild type (WT), LS1 $\Delta$ *sae*- or LS1 $\Delta$ *sarA*-mutant one week after surgery. 10 days later the mice were euthanized and kidneys, bones and catheters were removed for CFU counting (animal experiments were approved (LANUV, approval no. 81-02.04.2018.A334). The *in vitro* biofilm formation of strains was determined by crystal violet staining and the interaction of bacteria and host cells (primary endothelial cells, neutrophils) was analyzed.

**Results:** *In vitro* *S. aureus* LS1 WT and LS1 $\Delta$ *sae* could form biofilm, LS1 $\Delta$ *sarA* however not. Our *in vivo* model showed a completely different picture: Although the *sae* null-mutant was able to colonize the kidneys and bones, meaning that it was not immediately removed from the host, it was less able to colonize the intravascular catheter than the other strains. Subsequent *in vitro* experiments showed that the  $\Delta$ *sae*-mutant was less able to invade host cells and did not induce a proinflammatory response from neutrophils or HUVECs. In

contrast to LS1 WT and LS1 $\Delta$ *sarA*, LS1 $\Delta$ *sae* had no effect on the viability of neutrophils and could not cause a significant increase in neutrophil transmigration via a HUVEC cell layer compared to PBS control.

**Conclusion:** The result of biofilm formation *in vitro* did not reflect biofilm formation *in vivo* under the stress of the immune system. Although the  $\Delta$ *sarA*-mutant and wild type strain induced a host response that should help the host to eliminate the bacterial infection, this response seemed to enhance the bacteria's ability to form a biofilm.

## 163-MPV

### Group A streptococcal pyrogenic exotoxin B (SpeB) responses to intracellular environment in necrotizing skin and soft tissue infections

\*P. Shumba<sup>1</sup>, T. Sura<sup>2</sup>, J. Hoßmann<sup>3</sup>, D. Becher<sup>2</sup>, N. Siemens<sup>1</sup>

<sup>1</sup>Universität Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

<sup>2</sup>Universität Greifswald, Department of Microbial Proteomics, Greifswald, Germany

<sup>3</sup>Helmholtz Center for Infection Research, Microbial Interactions and Processes Research Group, Braunschweig, Germany

## Introduction

It is widely accepted that immune cells play a crucial role in hyper-inflammatory and tissue destructive processes of group A streptococcal (GAS) necrotizing skin and soft tissue infections (NSTIs). Several immune cells are recruited to the site of infection, including neutrophils and macrophages. Analyses of human NSTI biopsies revealed that high amounts of viable bacteria were readily detectable, even in those patients with prolonged antibiotic therapy. GAS secrete a potent cysteine protease streptococcal pyrogenic exotoxin B (SpeB). Mixed populations of SpeB-positive and negative GAS clones were identified in patient biopsies.

## Objectives

Here, we aimed to identify host factors that interfere with GAS SpeB expression.

## Materials & methods

To identify host factors responsible for a mixed SpeB phenotype within the biopsies, microscopic analyses of the tissue were performed. In addition, whole genome and proteome analyses of GAS NSTI isolates were conducted. Furthermore, the impact of human primary cells on GAS SpeB expression was analysed.

## Results

Although up-to 90% of GAS genome was identical between different GAS NSTI isolates, a diverse expression pattern of SpeB during the infection was detected. Human macrophages and neutrophils were identified as major sources driving the on/off switch of SpeB. However, this phenomenon was more pronounced in neutrophils. Further, *in vitro* passaging of SpeB-negative GAS clones revealed that the switch occurs mainly on the proteome level and not, as previously reported, on the genome level.

## Conclusion

Our data suggest that the on/off-set of SpeB expression potentially promotes intracellular survival in phagocytes. It is a reversible process, which suggests that GAS have adapted strategies to sense potential danger signals during an infection. We are currently addressing the survival strategies by using whole proteome analyses.

## 164-MPV

### Describing the Interaction between *Bartonella* Adhesin A and Fibronectin as a Novel Therapeutic Concept to Treat Bacterial Infections

\*D. J. Vaca<sup>1</sup>, A. Thibau<sup>1</sup>, M. S. Leisegang<sup>2</sup>, J. Malmström<sup>3</sup>, L. Happonen<sup>3</sup>, V. A. J. Kemp<sup>1</sup>

<sup>1</sup>Goethe University of Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt am Main, Germany

<sup>2</sup>Goethe University of Frankfurt, Institute for Cardiovascular Physiology, Frankfurt am Main, Germany

<sup>3</sup>Lund University, Division of Infection Medicine, Lund, Sweden

**Introduction:** *Bartonella henselae* is a facultative intracellular bacterium, responsible for cat scratch disease and vascular proliferations (bacillary angiomatosis) in humans. The trimeric autotransporter adhesin *Bartonella* adhesin A (BadA) is a major pathogenicity factor of *B. henselae* mediating bacterial adherence to endothelial cells (EC) and extracellular matrix (ECM) proteins. The identification of specific binding sites between BadA and ECM proteins might give insights about the use of BadA-specific peptides to treat bacterial infections by a new class of antibiotics ("anti-ligands").

**Objective:** To describe BadA binding sites in fibronectin (FN) as the basis of the interaction between BadA and ECM proteins in host-cell adhesion.

**Material & Methods:** *B. henselae* strains (wild type and BadA deficient) were exposed to proteolytic FN fragments to analyze binding affinity via ELISA and western blot. Competition assays were performed using bacteria and heparin or antibodies targeting specific FN regions. The BadA-FN interaction was further analyzed by mass spectrometry of cross-linked peptides. As a proof of concept for the importance of BadA-FN interaction in the infection process, FN deficient ECs (LentiCRISPR) were generated and incubated with different FN fragments and *B. henselae* strains using *in vitro* infection models.

**Results:** Binding experiments between *B. henselae* and FN fragments were performed showing higher affinity of BadA to a 30 kDa fragment located at the N-terminus and 40 kDa at the C-terminus of the FN molecule. This interaction was supported by mass spectrometry analysis of the cross-linked peptides and by the binding inhibition after competition assays with heparin. The importance of this interaction was evaluated via infection experiments. After the definition of BadA-FN binding sites, further steps will include the generation of synthetic molecules for the inhibition of bacterial adhesion to host cells ("anti-ligands").

**Conclusions:** The two heparin-binding domains of the FN molecule were identified as the major BadA binding sites. The role of these binding sites will be analyzed in the course of infection using *in vitro* infection assays.

---

## 165-MPV

Media-mediated difference in virulence and resistance of *Pseudomonas aeruginosa*

\*V. Behrends<sup>1</sup>

<sup>1</sup>University of Roehampton, Life Sciences, London, United Kingdom

**Introduction** - *Pseudomonas aeruginosa* is one of the most important opportunistic, nosocomial pathogens. It has an impressive range of potential host species, as it is able to infect plants, invertebrates and vertebrates. The latter include (immunocompromised) humans, e.g. during chronic infections of the cystic fibrosis lung. This host range requires a large metabolic capacity and a high number of virulence determinants.

**Objectives** - This study aimed to investigate the interactions between metabolism and virulence over hundreds of generation of directed evolution in *P. aeruginosa*.

**Methods** - We evolved *P. aeruginosa* in 96 different growth conditions (BIOLOG GenIII plate) over the course of 300-400 generation in the presence or absence of sub-inhibitory concentrations of the antibiotics Ciprofloxacin and Tobramycin. Aliquots were frozen daily to trace evolutionary trajectories.

**Results** - We found that media composition influenced virulence potential (measured as ability to kill mammalian cells) both immediately and over longer time-scales. Interestingly, resistance patterns suggested media adaptation-mediated development of resistance (to Kanamycin) and development of cross-resistance in Ciprofloxacin-exposed bacteria. Virulence factors such as pyocyanin as well as quorum sensing signals showed a clear downward trend in rich media, suggesting the emergence of social cheaters in these conditions.

**Conclusion** - Our findings show that adaptation to media, both short and long-term has a clear impact on virulence potential, resistance and - unsurprisingly - growth of *P. aeruginosa*. The mechanisms behind these differences could be used to explore novel treatment options aiming to down-regulated virulence in the clinical setting.

---

## 166-GIV

Genome characterization of two multidrug-resistant *Arcobacter butzleri* isolates and prevalence of antibiotic resistance genes in strains isolated from poultry in Ghana

\*A. E. Zautner<sup>1</sup>, K. G. Boahen<sup>2</sup>, C. W. Akenten<sup>2</sup>, T. Riedel<sup>3</sup>, J. Overmann<sup>3</sup>, J. May<sup>4</sup>, D. Dekker<sup>4</sup>

<sup>1</sup>Universitätsmedizin Göttingen, Medizinische Mikrobiologie, Göttingen, Germany

<sup>2</sup>Kumasi Center for Collaborative Research in Tropical Medicine (KCCR), Kumasi, Ghana

<sup>3</sup>Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany

<sup>4</sup>Bernhard Nocht Institute for Tropical Medicine, Infectious Disease Epidemiology, Hamburg, Germany

In recent years, *Arcobacter butzleri* has gained notoriety as an emerging pathogen associated with poultry and water. *A. butzleri* has been detected in about 11% of diarrhea patients in South Africa. It may therefore be one of the main pathogens for diarrhea in other African countries, but this is relativized by the variable virulence of the individual isolates.

The aim of the study was (i) to identify antibiotic resistance genes (ARGs) in generated genome sequences of two multidrug-resistant *A. butzleri* isolates, (ii) to use multilocus sequence typing (MLST) to generate a guiding phylogeny of *A. butzleri* isolates from poultry in Ghana, (iii) to examine the distribution of ARGs in the test cohort, and (iv) to assess the virulence and the possible antibiotic treatment options for arcobacteriosis based on the genome sequences and the ARG distribution.

A total of 48 *A. butzleri* isolates were isolated from poultry distributed in Ghana. These isolates were MLS-typed and investigated by agar disk diffusion for their susceptibility to ciprofloxacin, tetracycline, gentamicin and erythromycin. Whole genome sequence data of two *A. butzleri* isolates were obtained by a combination of single-molecule real-time (SMRT) and Illumina sequencing technology. In the test cohort, the distribution of a total of 14 ARGs was investigated by amplicon sequencing.

With 43 different sequence types for 48 isolates, the test cohort proved to be phylogenetically very heterogeneous. A total of 14 ARGs and virulence factors similar to those of *Campylobacter* were identified in the two generated genome sequences. In total, only four isolates show an ARG constellation indicating a multidrug-resistant phenotype, while *oxa-464*, *macA* and *macB* and the quinolone determining region (QRDR) were present in the majority of isolates.

The evaluation of the virulence genes in the two *A. butzleri* genomes shows that the species may have a similar virulence potential as *Campylobacter* species.

The presence of the ARGs *oxa-464*, *macA* and *macB* and the QRDR in the predominant number of isolates indicates that ampicillin, macrolides and quinolones should not be recommended as antibiotic therapy for arcobacteriosis in Ghana.

---

## 167-GIV

### New sustainable therapeutic options for *Clostridioides difficile* therapy

\*M. Brauer<sup>1,2</sup>, S. Wagner<sup>3</sup>, J. Herrmann<sup>4</sup>, S. Sievers<sup>1</sup>, D. Zühlke<sup>1</sup>, V. I. Pauker<sup>1</sup>, R. Müller<sup>4</sup>, T. M. Fuchs<sup>3</sup>, K. Riedel<sup>1,2</sup>

<sup>1</sup>University of Greifswald, Institute of Microbiology, Greifswald, Germany

<sup>2</sup>Institute of Marine Biotechnology, Greifswald, Germany

<sup>3</sup>Federal Research Institute for Animal Health, Institute of Molecular Pathogenesis, Jena, Germany

<sup>4</sup>Helmholtz Centre for Infection Research and Pharmaceutical Biotechnology, Helmholtz Institute for Pharmaceutical Research Saarland, Department Microbial Natural Products, Saarbrücken, Germany

*Clostridioides difficile* is an important nosocomial pathogen causing gastrointestinal disease in patients who have received antimicrobial therapy recently. While people with an intact intestinal microbiome show colonization resistance towards *C. difficile*, spores of *C. difficile* are able to germinate in the gut of patients with a dysfunctional microbiome. Thus, antibiotics play a dual role in *C. difficile* infections by paving the way for the infection as well as by still being first-choice therapy.

With regard to recurrence rates of up to 30 %, we aim to characterize new antimicrobials for *C. difficile* therapy that show activity against *C. difficile* but ideally omit most other bacterial species in the gut.

To do so, we first performed broth dilution assays to identify compounds with low minimal inhibitory concentrations for *C. difficile* from a selection of antimicrobials that have been isolated from microorganisms. Subsequently, broth dilution assays were repeated to test for the action of the most active compounds on selected commensals from the gut. In parallel, proteomics experiments in *C. difficile* strain 630 were performed to gain information on the mode-of-action of the same compounds. Moreover, we also fed one compound, Chlorotoni A, to piglets and analyzed its impact on the intestinal microbiota using a multi-omics approach.

Our results show that the antimicrobial compounds Chlorotoni A and B, Chelocardin and Myxopyronin B are significantly active against *C. difficile* with minimal inhibitory concentrations below 3 µg/ml. First results from our proteomics experiments revealed that Chelocardin interacts with the bacterial cell envelope. Results for Chlorotoni A/B and Myxopyronin B are expected soon. In addition, the animal experiment and broth dilution assays for the

commensal bacteria showed that Chlorotoni A has a rather narrow spectrum activity affecting mainly groups of Gram-positive bacteria, e.g. families within the order Clostridiales. Therefore, we hypothesize that Chlorotoni A and B could be promising candidates in *C. difficile* therapy. Future infection experiments in mice will help to further validate this hypothesis.

---

## 168-GIV

### IFN-STAT1-MLKL axis drives necroptosis during gastrointestinal infection by controlling host cell death

\*I. Stolzer<sup>1</sup>, M. T. Chiriac<sup>1</sup>, M. F. Neurath<sup>1</sup>, C. Günther<sup>1</sup>

<sup>1</sup>Universitätsklinikum Erlangen, Department of Medicine 1, Erlangen, Germany

The invasive bacterium *Salmonella enterica* is a common gastrointestinal pathogen and causes severe foodborne illness worldwide. Intestinal homeostasis and the maintenance of the epithelial barrier are essential components of host defense during gastrointestinal infection and requires a strict regulation of cell death. Interferons (IFNs) are potent immune-modulatory cytokines that are strongly expressed by several cell types in response to inflammation and infection. It has just recently been reported that IFNs potentially influence the pathogenesis of several inflammatory diseases by mediating regulated necrosis by altering the expression of *Mkl* via STAT1. However, molecular mechanisms of cell death regulation are not fully understood. Thus, we elucidated the contribution of different forms of cell death and upstream regulatory components. An IFN-STAT1 axis ensures host defense during *Salmonella* Typhimurium infection by limiting infected cells either by Caspase8-mediated apoptosis or MLKL-mediated necroptosis. A strict cell death regulation is nevertheless required since mice lacking Caspase8, an essential regulator of apoptosis and negative regulator of necroptosis, are highly sensitive towards gastrointestinal infection and inflammation associated with lethality. Our results demonstrate that epithelial STAT1 signaling controls *Mkl* gene expression in intestinal epithelial cells during *Salmonella* Typhimurium infection and that STAT1 signaling plays a crucial role for the course of disease based on maintaining intestinal homeostasis and for host survival. This pathway seems to be pathogen specific since STAT1 has only a minor contribution to coordinate cell death during other gastrointestinal infections. Furthermore, we suppose a contribution of STAT2 beside STAT1 to maintain intestinal homeostasis and epithelial integrity during inflammation and infection. Collectively, our study reveals that IFN-STAT-signaling maintains homeostasis of the intestinal barrier by altering cell death regulatory components. Our data suggest that IFNs and epithelial STAT1-signaling maintains intestinal tissue homeostasis during *Salmonella* infection by controlling host cell death.

---

## 169-GIV

### The HopQ-CEACAM interaction controls CagA translocation, phosphorylation and phagocytosis of *Helicobacter pylori* in neutrophils

\*I. K. Behrens<sup>1</sup>, B. Busch<sup>1</sup>, P. Palamides<sup>1</sup>, J. E. Shively<sup>2</sup>, C. Stanners<sup>3</sup>, C. Chan<sup>4</sup>, N. Leung<sup>5</sup>, S. Gray-Owen<sup>5</sup>, R. Haas<sup>1</sup>

<sup>1</sup>Max von Pettenkofer Institute, Chair of Medical Microbiology and Hospital Epidemiology, LMU, Munich, Germany

<sup>2</sup>Beckman Research Institute, Department of Molecular Imaging and Therapy, City of Hope, Duarte, United States

<sup>3</sup>McGill University, Department of Biochemistry and Goodman Cancer Research Centre, Montreal, Quebec, Canada

<sup>4</sup>University of Iowa Carver College of Medicine, Division of Surgical Oncology and Endocrine Surgery, Iowa City, United States

*Helicobacter pylori* avoids its elimination by the host immune system and persistently colonizes approximately 50% of the world population. This is accompanied by a strong infiltration of neutrophil granulocytes resulting in a bacterial gastritis that may lead to peptic ulcer disease, duodenal ulcer, MALT (mucosa-associated lymphoid tissue) lymphoma or gastric cancer. A hallmark of the human pathogen is its *cag* type IV secretion system (*cag*-T4SS). Translocation of the oncoprotein CagA of *H. pylori* via the *cag*-T4SS is facilitated by carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), which interact with the *Helicobacter* outer membrane protein Q (HopQ) of *H. pylori*. In this work the interaction of *H. pylori* with human CEACAM1, CEACAM3 and CEACAM6 receptors (hCEACAMs) expressed on PMNs from humanized mice was investigated. It could be shown that human CEACAMs strongly enhance HopQ-dependent the amount of translocated CagA. Afterwards, CagA gets tyrosine phosphorylated in human PMNs. In this work the influence of the CEACAM-HopQ interaction on tyrosine-phosphorylation of CagA was shown. In addition, it could be demonstrated that CEACAMs change the functions of neutrophils in a *H. pylori* infection. CEACAMs enhance the production of reactive oxygen species and play a special role during phagocytosis. Moreover, the release of proinflammatory chemokines was affected by the *H. pylori* CEACAM interaction. Taken together, this work demonstrates the importance of the HopQ-CEACAM interaction on neutrophils for *H. pylori* and might lead to a better understanding of the host pathogen interaction.

## 170-GIV

Deciphering the bewildering variety of O-glycans derived from mammalian mucins by state-of-the-art mass spectrometry

\*M. Mormann<sup>1</sup>, G. Pohlentz<sup>2</sup>, S. Kruse<sup>1</sup>, I. Yilmaz<sup>1</sup>, J. Mühling<sup>2</sup>

<sup>1</sup>University of Münster, Institute of Hygiene, Biomedical Mass Spectrometry Group, Münster, Germany

<sup>2</sup>University of Münster, Institute of Hygiene, Glycobiology Group, Münster, Germany

**Introduction:** Mucus glycoproteins, i.e. mucins constitute as major components the mucosal protective barrier of the gastrointestinal tract. Mucins are densely decorated with O-glycans exhibiting a vast structural diversity. Specific glycan motifs play key roles in mucosal protection and also in extrinsic interaction with commensal and pathogenic bacteria. To this day, there is a substantial lack of knowledge of the precise structures of the glycans from mucin glycoproteins.

**Objective:** To decipher the heterogeneity of O-glycans derived from mammalian mucins an approach involving a novel sample preparation protocol combined with state-of-the-art mass spectrometry (MS) techniques is employed.

**Materials and Methods:** Mucins obtained from porcine stomach and from bovine maxillary gland served as model substances. O-glycans were chemically liberated from the protein backbone by use of  $\beta$ -elimination. Released carbohydrates were characterized by MS<sup>1</sup>- and MS<sup>2</sup>-analysis including collision-induced dissociation (CID) and ion mobility MS.

**Results:** The release of O-glycans from the protein backbone of mucin from porcine stomach by  $\beta$ -elimination reactions in combination with MS analysis revealed heterogeneous mixtures of more than 35 distinct neutral

glycoforms harboring core type 1 and type 2 structures. Besides simple di- and trisaccharides complex fucosylated oligosaccharides up to octadecamers were found and their structures were elucidated by low-energy CID experiments. Chemical liberation of O-glycans from mucin of bovine submaxillary glands yielded a high number of differently fucosylated and sialylated glycoforms based on core 2 and core 3 structures. Taking advantage of the various collisional cross-sections of isomeric oxonium-type fragment ions allowed discriminating different linkage types of terminal sialic acids.

**Conclusion:** The present results demonstrate the potential of MS analysis of O-glycans released from mammalian mucins for their in-depth structural characterization. Knowledge on the exact glycan structure is imperative for understanding the functional role of O-glycans in the interaction of resident and harmful microorganisms with the mucosal barrier.

## 171-GIV

### Proteoglycan-Dependent Endo-Lysosomal Fusion Affects Intracellular Survival of *Salmonella typhimurium* in Epithelial Cells

\*A. Galeev<sup>1</sup>, A. Suwandi<sup>1</sup>, A. Oktiviyari<sup>1</sup>, H. Bakker<sup>2</sup>, F. Routier<sup>2</sup>, M. Hensel<sup>3</sup>, G. A. Grassl<sup>1</sup>

<sup>1</sup>Hannover Medical School and German Center for Infection Research (DZIF), Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany

<sup>2</sup>Hannover Medical School (MHH), Institute of Clinical Biochemistry, Hannover, Germany

<sup>3</sup>University of Osnabrueck, Division of Microbiology, Osnabrück, Germany

## Introduction

Cell surface proteoglycans (PGs) are glycosylated proteins involved in cell-matrix and cell-cell interactions which also play an important role in bacterial adhesion, invasion, and immune response. PGs consist of a core protein linked to chains of glycosaminoglycans (GAGs), such as chondroitin sulphate, heparan sulphate, and heparin. The initial step of GAG assembly is facilitated by the  $\beta$ -D-xylosyltransferase enzyme encoded by the *XylT2* gene.

## Objective

To elucidate a role of the PGs in *Salmonella Typhimurium* (*S. Typhimurium*) infection.

## Methods

Gentamicin protection assay was employed to assess an invasion into and replication of *S. Typhimurium* in wild-type and xylosyltransferase-deficient ( $\Delta$ XylT2) Chinese hamster ovary (CHO) cells. Gentamicin concentration in the CHO cells was measured by ELISA. *S. Typhimurium* reporter strains were used to determine the intracellular localization of *Salmonella*.

## Results

*S. Typhimurium* adhered to and invaded into CHO WT and CHO  $\Delta$ XylT2 cells at comparable levels. However, 24 hours after infection, proteoglycan-deficient CHO cells were significantly less colonized by *S. Typhimurium* compared to CHO WT. This proteoglycan-dependent phenotype was rescued by addition of GAGs (heparin) to the medium, as well as by complementation of the *XylT2* gene. No



differences in the levels of intracellular gentamicin concentration between CHO WT and CHO  $\Delta$ XylT2 cells were detected. Chloroquine resistance assay and immunostaining revealed that in the absence of PGs, significantly less bacteria were associated with *Salmonella*-containing vacuoles (SCVs) when compared to WT CHO cells. Notably, cytosolic bacteria had similar replication rates in CHO WT and CHO  $\Delta$ XylT2 cells. Inhibition of endo-lysosomal fusion by a specific inhibitor of phosphatidylinositol phosphate kinase PIP5KIII (PIKfyve) significantly increased *Salmonella* burdens in CHO  $\Delta$ XylT2 cells, but decreased *Salmonella* loads in CHO WT cells, demonstrating an importance of PGs for membrane-associated intracellular bacteria.

## Conclusion

Proteoglycans influence survival of *Salmonella* at the later time points of infection via an action of PIKfyve-dependent endo-lysosomal fusion.

## 172-GIV

Application of NGS as method for typing of *Salmonella enterica* serovar Typhi

\*E. Trost<sup>1</sup>, S. Simon<sup>1</sup>, S. Fuchs<sup>2</sup>, A. Fruth<sup>1</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>Robert Koch-Institut, Division of Enteropathogenic Bacteria and Legionella (FG11), Wernigerode, Germany

<sup>2</sup>Robert Koch-Institut, Unit 13: Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany

Since 1938, isolates of *S. Typhi* are classified by phage typing, based on lysis of bacterial strains by specific bacteriophages. The set comprises 110 Vi-adapted phages [1].

We describe the sequencing and analysis of *S. Typhi* isolates sent to the NRC from 2015 to 2019. Our aim is to establish NGS as a routine typing method. Therefore we evaluate SNP- and cgMLST-based approaches and compare it with classical typing methods.

SNP-based phylogenetic analyses were done with Geneious. SeqSphere+ was applied for cgMLST analysis based on strain CT18 and the Enterobase wgMLST scheme [6]

1. *S. Typhi* isolates classified as ubII are not lysed by Villi-typing phages, preventing a reliable statement about epidemiological contexts. In these cases NGS based methods showed a high degree of differentiation.

NGS data were used to identify isolates of lineage H58 and XDR, characterized by 44 SNPs and multiple antibiotic resistances. We were able to identify these SNPs in 114 isolates including 5 XDR isolates. Both, SNP- and cgMLST-based analyses were successfully applied as routine methods and outbreak detection.

1. Rabsch, W., et al., *Typing phages and prophages of Salmonella*, in *In Salmonella: From Genome to Function*. 2011, Ed. S. Porwollik, Caister Academic Press: Norfolk, UK. p. 25-48.
2. Parkhill, J., et al., *Complete genome sequence of a multiple drug resistant Salmonella enterica serovar Typhi CT18*. *Nature*, 2001. **413**(6858): p. 848-52.
3. Langmead, B. and S.L. Salzberg, *Fast gapped-read alignment with Bowtie 2*. *Nat Meth*, 2012. **9**(4): p. 357-359.
4. Li, H., *A statistical framework for SNP calling, mutation discovery, association mapping and*

*population genetical parameter estimation from sequencing data*. *Bioinformatics*, 2011. **27**(21): p. 2987-93.

5. Koboldt, D.C., et al., *VarScan: variant detection in massively parallel sequencing of individual and pooled samples*. *Bioinformatics*, 2009. **25**(17): p. 2283-5.
6. Alikhan, N.F., et al., *A genomic overview of the population structure of Salmonella*. *PLoS Genet*, 2018. **14**(4): p. e1007261.

## 173-GIV

Early *in vivo* genome and methylome adaptation of the *cag*-negative *Helicobacter pylori* strain BCS 100 during an experimental human infection study

\*I. Estibariz<sup>1,2</sup>, F. Ailloud<sup>1</sup>, S. Woltemate<sup>3</sup>, B. Bunk<sup>4</sup>, C. Spröer<sup>4</sup>, J. Overmann<sup>4</sup>, T. Aebischer<sup>5,6</sup>, T. F. Meyer<sup>5</sup>, C. Josenhans<sup>1,3,2</sup>, S. Suerbaum<sup>1,3,2</sup>

<sup>1</sup>Max von Pettenkofer Institute - LMU Munich, Med. Microbiology and Hospital Epidemiology, München, Germany

<sup>2</sup>DZIF - German Center for Infection Research, München, Germany

<sup>3</sup>MHH Hannover Medical School, Med. Microbiology and Hospital Epidemiology, Hannover, Germany

<sup>4</sup>DSMZ, Braunschweig, Germany

<sup>5</sup>Max Planck Institute for Infection Biology, Berlin, Germany

<sup>6</sup>Robert Koch Institute, Berlin, Germany

*Helicobacter pylori* has been shown to undergo rapid *in vivo* evolution in the course of chronic infection. In contrast, little is known about genome and methylome variation during the first stages of the infection. Here, we investigated genome and methylome evolution of *H. pylori* strain BCS 100 in 10 human volunteers. The *cag*PAI-negative *H. pylori* strain BCS 100 is the first strain that has been used in multiple challenge trials involving human volunteers. Genome sequences were generated for 16 *H. pylori* clones isolated from the challenge strain, and from 20 clones re-isolated from the antrum and corpus of 10 human volunteers who participated in a *H. pylori* challenge study to evaluate a recombinant *Salmonella*-based vaccine candidate. Comparison of whole genome sequences revealed genomic variability within the challenge strain population, and provided evidence of adaptation to the individual volunteers, affecting mostly genes with an outer-membrane related role and genes involved in peptide uptake. One re-isolate displayed a duplication of the catalase gene, which might help with fighting oxidative stress in an individual with a high degree of inflammation. Methylome differences were due to the ON/OFF switching of the enzymatic activity of phase-variable MTase genes. Adaptation of *H. pylori* to different individuals and stomach niches with particular selective pressures included genome and methylome modifications specific to every isolate. Thus, *H. pylori* is a versatile bacterium able to adapt to new hosts via changes in several genes and, probably, by modifying gene expression due to changes in the epigenome.

## 174-III

Tracking of influenza-specific memory B-lymphocytes in blood by single cell analysis and deep sequencing of the variable B-cell receptor regions

\*C. Jassoy<sup>1</sup>, S. Reiche<sup>1</sup>, S. Horn<sup>1</sup>, Y. Dwai<sup>1</sup>

<sup>1</sup>Universität Leipzig, Institut für Virologie, Leipzig, Germany

**Background and Aim:** Viral infection and vaccination induce antigen-specific memory B-cell responses. The aim of the study was to identify and subsequently track influenza nucleoprotein (NP)-specific memory B-cells in the blood of an individual by single cell analysis and deep sequencing.

**Methods:** Influenza NP-specific memory B-lymphocytes were isolated from blood at three time points over 19 months. BcR IgG heavy and light chain variable regions of single antigen-specific memory B-cells were amplified by RT-PCR, cloned in antibody expression plasmids and sequenced. Antibody function was determined by ELISA. At the last blood drawing, the heavy chain regions were additionally analysed by MiSeq deep sequencing. Amino acid sequences from single cell analysis and deep sequencing were compared.

**Results:** Single cell BcR gene amplification from memory B-cells and cloning of heavy and light antibody chains yielded 26 structurally different influenza NP-specific antibodies. Three of the BcR sequences of single memory B-cells were also found by deep sequencing 15 to 19 months later.

**Conclusion:** By using antigen-specific memory B-cell isolation and deep sequencing, influenza NP-specific memory B-lymphocytes are traceable in the blood for more than a year. Deep sequencing can possibly be used to longitudinally assess the antigen-specific memory B-cell repertoire and to determine the durability of memory B-cell clones in vivo.

---

#### 175-III

Single-cell RNA-seq reports growth condition-specific global transcriptomes of individual bacteria

\*J. Vogel<sup>1</sup>

<sup>1</sup>Helmholtz Institute, Institute for RNA-based Infection Research, Würzburg, Germany

Single-cell transcriptomics is revolutionizing the analysis of phenotypic cell-to-cell variation in eukaryotes but technical hurdles have prevented its robust application to prokaryotes. Using poly(A)-independent single-cell RNA-seq, we report the faithful capture of growth-dependent gene expression patterns in individual *Salmonella* and *Pseudomonas* bacteria, across all RNA classes and genomic regions. These transcriptomes provide important reference points for single-cell RNA-seq of other bacterial species, mixed microbial communities and host-pathogen interactions.

---

#### 176-III

##### Modulation of macrophage transcription and epigenetics by *Yersinia enterocolitica*

\*I. Bekere<sup>1</sup>, J. Huang<sup>1</sup>, M. Schnapp<sup>1</sup>, M. Aepfelbacher<sup>1</sup>

<sup>1</sup>University Medical Center Hamburg-Eppendorf (UKE), Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany

The pathogenic *Yersinia pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* suppress the host immune response by downregulating the transcription of inflammatory genes. This activity is encoded on a virulence plasmid which allows the bacteria to inject effector proteins (Yops) into the host cells via the bacterial type three secretion system. We investigated whether epigenetic histone modifications play a role in the global effect of *Y. enterocolitica* on gene transcription. Therefore we infected primary human macrophages with *Y. enterocolitica* strains (wild type,  $\Delta$ YopM and  $\Delta$ YopP mutants and the virulence plasmid deficient WAC strain) and performed Chromatin Immuno-Precipitation (ChIP)-seq employing anti-H3K4me3, -H3K27ac, -H3K27me3 and -H3K4me1 antibodies in combination with RNA-seq. Wild type induced activating histone marks at ~2100 genes at promoters, which dose dependently paralleled transcription. Further, wild type *Yersinia* strongly modulated activating H3K27ac at enhancers of more than

5000 genes. Integration of all data revealed that wild type upregulated promoters and enhancers of genes for metabolism and small Rho GTPase signaling. In addition, wild type suppressed activation of promoters and enhancers of immune response genes. YopM did not to play a significant role while YopP showed a major contribution to the wild type effects, suggesting that Yops other than YopP or YopM or the interplay of all Yops contribute to this phenomenon. Moreover, the effect of YopP on the chromatin is partially mediated through inhibition of the MAPK pathway. In summary, we provide evidence for a profound epigenetic and transcriptional reprogramming of macrophage metabolism, small Rho GTPase signaling and inflammatory response by virulent *Y. enterocolitica*.

---

#### 177-III

##### Plasma Kallikrein as a novel host pathogenicity factor in *Streptococcus pyogenes* sepsis

\*J. Köhler<sup>1</sup>, C. Maletzki<sup>2</sup>, D. Koczan<sup>3</sup>, M. Frank<sup>4</sup>, C. Trepesch<sup>5,1</sup>, A. S. Revenko<sup>6</sup>, J. R. Crosby<sup>6</sup>, A. R. M. Macleod<sup>6</sup>, S. Mikkat<sup>7</sup>, S. Oehmcke-Hecht<sup>1</sup>

<sup>1</sup>Rostock University Medical Center, Institute of Medical Microbiology, Virology and Hygiene, Rostock, Germany

<sup>2</sup>Rostock University Medical Center, Department of Internal Medicine, Medical Clinic III - Hematology, Oncology, Palliative Care, Rostock, Germany

<sup>3</sup>Rostock University Medical Center, Center for Medical Research – Core Facility Micro-Array-Technologie, Rostock, Germany

<sup>4</sup>Rostock University Medical Center, Medical Biology and Electron Microscopy Centre, Rostock, Germany

<sup>5</sup>Charité – Universitätsmedizin Berlin, Department of Anesthesiology and Operative Intensive Care Medicine, Berlin, Germany

<sup>6</sup>Ionis Pharmaceuticals, Inc., Department of Antisense Drug Discovery, Carlsbad, United States

<sup>7</sup>Rostock University Medical Center, Core Facility Proteome Analysis, Rostock, Germany

#### Introduction

Sepsis causes an activation of the human contact system, an inflammatory response mechanism against foreign surfaces, proteins and pathogens. The serine proteases of the contact system, factor XII and plasma kallikrein, are decreased in plasma of septic patients, which was previously associated with an unfavorable outcome. However, the precise mechanisms and roles of contact system factors in bacterial sepsis are poorly understood.

#### Objectives

We studied the physiological relevance of factor XII and plasma kallikrein in streptococcal infections.

#### Material & Methods

In this study, we used a subcutaneous infection model with *S. pyogenes* with and without pretreatment of ASOs through intraperitoneal injections. We analyzed bacterial dissemination, factor XII and prekallikrein gene (*klkb1*) expression, plasma kallikrein concentration in septic mice plasma and we did *in vitro* studies to the relevance of PK during fibrinolysis.

#### Results and Conclusion

Decreased plasma kallikrein concentration in septic mice is a result of reduced RNA expression of *klkb1* gene, indicating that plasma kallikrein belongs to negative acute phase proteins. Investigations regarding the pathophysiologic function of contact system proteases during sepsis revealed different roles for factor XII and plasma kallikrein. *In vitro*,

factor XII decelerated bacteria induced fibrinolysis, whereas plasma kallikrein supported it. Depletion of plasma kallikrein – but not factor XII – by treatment with ASOs, dampens bacterial dissemination and growth in multiple organs in the mouse sepsis model. These findings identify plasma kallikrein as a novel host pathogenicity factor in *Streptococcus pyogenes* sepsis.

#### 178-IIV

The author has not agreed to a publication.

#### 179-IIV

Characterization of *Staphylococcus aureus* isolated from hyper-IgE syndrome patients by whole genome sequencing

\*V. Schwierzeck<sup>1</sup>, R. Effner<sup>2</sup>, F. Abel<sup>2,3</sup>, M. Reiger<sup>2</sup>, G. Notheis<sup>2</sup>, A. de Tomassi<sup>2</sup>, J. Held<sup>4</sup>, V. Simon<sup>5</sup>, S. Dinter<sup>6</sup>, S. Stentzel<sup>7</sup>, B. Bröker<sup>7</sup>, R. Hoffmann<sup>5</sup>, J. Hübner<sup>3</sup>, B. Hagel<sup>2</sup>, A. Mellmann<sup>1</sup>, C. Traidl-Hoffmann<sup>2,8</sup>, E. D. Renner<sup>2,8</sup>

<sup>1</sup>Institute for Hygiene Münster, University Hospital Münster, Münster, Germany

<sup>2</sup>Environmental Medicine, UNIKA-T, Technical University of Munich and Helmholtz Zentrum Munich, Munich/Augsburg, Germany

<sup>3</sup>University Children's Hospital, Dr. von Haunersches Kinderspital, Ludwig Maximilian University, Munich, Germany

<sup>4</sup>Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Universitätsklinikum Erlangen und Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Erlangen, Germany

<sup>5</sup>Institute for Laboratory Medicine and Microbiology, University Hospital Augsburg, Augsburg, Germany

<sup>6</sup>Institute of Pathology, University Hospital Augsburg, Augsburg, Germany

<sup>7</sup>Institute of Immunology and Transfusion Medicine, University Medicine Greifswald, Greifswald, Germany

<sup>8</sup>Hochgebirgsklinik and Christine-Kühne-Center for Allergy Research and Education, Davos, Switzerland

**Introduction:** Hyper-IgE syndromes (HIES) are a group of primary immunodeficiencies caused by monogenic defects such as in the gene *STAT3* (STAT3-HIES). Similar to patients with atopic eczema (AE), patients suffering from HIES show frequent *Staphylococcus aureus* (*S. aureus*) colonization. A recent study reported that American STAT3-HIES patients are often carriers of Panton-Valentine leukocidin (PVL) expressing, methicillin resistant *S. aureus* (MRSA) strains. So far, the epidemiology of *S. aureus* in German STAT3-HIES patient has not been studied yet.

**Objectives:** To characterize *S. aureus* strains isolated from STAT3-HIES and AE patients in Germany by whole genome sequencing (WGS) and to determine virulence gene repertoire.

**Materials and methods:** 11 *S. aureus* strains from STAT3-HIES patients (n=4) and 6 *S. aureus* strains isolated from AE patients (n=4) were analyzed by whole genome sequencing (WGS) and protein A (spa) typing.

**Results:** *S. aureus* strains isolated from our cohort of STAT3-HIES patients did not express PVL or enterotoxins and only one isolate was identified as MRSA. Spa types of collected isolates matched the molecular epidemiology of *S. aureus* in Germany (t015, t091, t084). Immune evasion cluster (IEC) genes, such as the chemotaxis inhibitory protein (chp) gene, were frequently detected in *S. aureus* isolates from both STAT3-HIES- and AE patients. One out of the four STAT3-HIES patients developed a lymph node abscess during the time of our study. Here WGS of *S. aureus* isolates illustrated that infection (lymph node abscess) and colonization occurred with different *S. aureus* strains instead of one single strain.

**Conclusion:** *S. aureus* strains isolated from our cohort of STAT3-HIES patients do not resemble *S. aureus* strains described by previous studies in regard to toxin expression, methicillin resistance and classified spa type. This study demonstrates how WGS can be used to characterize pathogens in the context of primary immunodeficiencies and improve our understanding of *S. aureus* infections in this patient group.

#### 180-IIV

##### NCX1-dependent Na<sup>+</sup> sensing represents a novel ionic danger amplification mechanism in macrophages

\*P. Neubert<sup>1</sup>, A. Homann<sup>1</sup>, D. Wendelborn<sup>1</sup>, A. L. Bär<sup>1</sup>, A. Schröder<sup>2</sup>, S. Ebner<sup>1,3</sup>, A. Weichselbaum<sup>1</sup>, V. Schatz<sup>1</sup>, P. Linz<sup>4</sup>, R. Veelken<sup>5</sup>, J. Schulte-Schrepping<sup>6</sup>, A. C. Aschenbrenner<sup>6,7</sup>, T. Quast<sup>6</sup>, C. Kurts<sup>8</sup>, S. Geisberger<sup>9</sup>, K. Kunzelmann<sup>10</sup>, K. Hammer<sup>11</sup>, K. J. Binger<sup>12</sup>, J. Titze<sup>13</sup>, D. N. Müller<sup>9</sup>, W. Kolanus<sup>6</sup>, J. L. Schultze<sup>6,14</sup>, S. Wagner<sup>11</sup>, J. Jantsch<sup>1</sup>

<sup>1</sup>Institute of Clinical Microbiology and Hygiene, University Hospital Regensburg, Regensburg, Germany

<sup>2</sup>Institute of Orthodontics, University Hospital Regensburg, Regensburg, Germany

<sup>3</sup>MPI of Biochemistry, Martinsried, Germany

<sup>4</sup>Institute of Radiology, University Hospital Erlangen, Erlangen, Germany

<sup>5</sup>Department of Internal Medicine 4, University Hospital Erlangen, Erlangen, Germany

<sup>6</sup>Department for Genomics & Immunoregulation, Life & Medical Sciences (LIMES) Institute, Bonn, Germany

<sup>7</sup>Department of Internal Medicine and Radboud Center for Infectious Diseases (RCI), Radboud University Medical Center, Nijmegen, Netherlands

<sup>8</sup>Institute of Experimental Immunology, University of Bonn, Bonn, Germany

<sup>9</sup>Experimental and Clinical Research Center, Max-Delbrück Center for Molecular Medicine and Charité-Universitätsmedizin, Berlin, Germany

<sup>10</sup>Institute of Physiology, University of Regensburg, Regensburg, Germany

<sup>11</sup>Department of Internal Medicine II, University Hospital of Regensburg, Regensburg, Germany

<sup>12</sup>Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, Bundoora, Australia

<sup>13</sup>Cardiovascular and Metabolic Disorders, Duke-NUS Medical School, Singapore, Singapore

<sup>14</sup>Platform for Single Cell Genomics & Epigenomics at the German Center for Neurodegenerative Diseases (DZNE), University of Bonn, Bonn, Germany

Inflammation and infection can trigger local tissue Na<sup>+</sup>-accumulation. This Na<sup>+</sup>-rich environment boosts pro-inflammatory activation of monocyte/macrophage-like cells and their antimicrobial activity. Enhanced Na<sup>+</sup>-driven macrophage function requires nuclear factor of activated T cells 5 (NFAT5)-dependent signaling, which augments NO production and contributes to increased autophagy. However, the mechanism of Na<sup>+</sup>-sensing in macrophages remained unclear. High extracellular Na<sup>+</sup> levels (HS) trigger a substantial Na<sup>+</sup>-influx with concomitant low Ca<sup>2+</sup> levels. We show here that the Na<sup>+</sup>/Ca<sup>2+</sup>-exchanger 1 (NCX1) plays a critical role in HS-triggered Na<sup>+</sup>-influx, concomitant Ca<sup>2+</sup> loss and subsequent HS-amplified antimicrobial macrophage activation. Interfering with NCX1-activity impairs osmoprotective inflammatory signaling, autolysosome formation and subsequent antibacterial activity of macrophages. Taken together, this demonstrates that NCX1-dependent sensing of the ionic environment is required for amplifying antimicrobial macrophage responses upon HS. Manipulating NCX1 offers a new strategy to manipulate macrophage function.

#### 181-IIV

The mitochondrial apoptosis apparatus contributes to the cell-autonomous defence in the absence of cell death

\*D. Brokatzky<sup>1</sup>, B. Dörflinger<sup>1</sup>, A. Haimovici<sup>1</sup>, A. Weber<sup>1</sup>, S. Kirschnek<sup>1</sup>, J. Vier<sup>1</sup>, A. Metz<sup>1</sup>, J. Henschel<sup>1</sup>, T. Steinfedt<sup>2</sup>, G. Häcker<sup>1</sup>

<sup>1</sup>Uniklinik Freiburg, IMMH, Freiburg i. Br., Germany

<sup>2</sup>Uniklinik Freiburg, Freiburg, Germany

Many pathogens have pro-apoptotic potential but apoptosis often occurs only in some conditions and on a small scale. It has recently become clear that the apoptotic signaling pathway can experimentally also be engaged at a low level, in the absence of cell death. We have found that such sub-lethal engagement of mitochondrial apoptosis-signaling occurs during infection of human epithelial cells by all tested viruses (HSV, Influenza A, MVA), bacteria (*Salmonella* Typhimurium, *Chlamydia trachomatis*) and one protozoon (*Toxoplasma gondii*). All of these infectious agents activated the mitochondrial apoptosis pathway without causing actual cell death, and all caused genomic DNA damage through this pathway. We have further found that low-level activation of the mitochondrial apoptosis apparatus is sufficient to induce cytokine secretion from epithelial cells, which was controlled by the Bcl-2-protein family (Bax, Bak, Bcl-XL). Different infectious agents induced the secretion of different cytokines. In all cases, mitochondrial apoptosis-signaling, triggered by the infection, significantly contributed to this cytokine secretion. When this signaling was experimentally blocked by the deletion of the essential apoptosis regulators Bax and Bak or by overexpression of Bcl-XL, intracellular growth of *Salmonella* and of *Chlamydia* was enhanced, suggesting that sub-lethal apoptosis signaling contributes to the cell-autonomous defence. The mitochondrial apoptosis apparatus thus has an independent, sub-lethal role in the host response to infection.

## 182-ZOV

Comparative transcriptomics combined with RNA interference reveals basis for differential cellular Shiga toxin sensitivity

\*A. Kehl<sup>1</sup>, I. U. Kouzel<sup>1</sup>, P. Berger<sup>1</sup>, D. Steil<sup>1</sup>, W. Makalowski<sup>2</sup>, Y. Suzuki<sup>3</sup>, H. Karch<sup>1</sup>, A. Mellmann<sup>1</sup>, J. Müthing<sup>1</sup>

<sup>1</sup>University of Muenster, Institute of Hygiene, Münster, Germany

<sup>2</sup>University of Muenster, Institute of Bioinformatics, Muenster, Germany

<sup>3</sup>University of Tokyo, Department of Computational Biology and Medical Sciences, Tokyo, Germany

## Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) are the main cause of the life-threatening hemolytic-uremic syndrome. The cardinal virulence factor is the Shiga toxin (Stx). Though kidney glomerular endothelial cells are the major target of Stx during pathogenesis, recent studies indicate that renal epithelial cells are susceptible to the cytotoxic action of Stx.

## Objectives

To analyze the Stx receptor, globotriaosylceramide (Gb3Cer), of a toxin-sensitive, ACHN, and a toxin-resistant, Caki-2, human kidney epithelial cell line, to explore the differences in transcriptomic responses to Stx2a and to characterize the results by RNA interference (RNAi).

## Methods

The cellular Gb3Cer profile was analyzed by mass spectrometry. RNAseq was applied to cells exposed to Stx2a. RNAi was employed targeting host factors involved in

Stx-related retrograde trafficking. Cytotoxicity was determined using the crystal violet assay.

## Results

ACHN and Caki-2 cells exhibited an opposite sensitivity towards Stx2a, even though they displayed a virtually identical Gb3Cer profile. Using comparative transcriptomics, we determined that ACHN and Caki-2 gene expression differed upon Stx2a exposure. The most differentially expressed genes are i.a. involved in the secretion of pro-inflammatory cytokines and retrograde trafficking of Stx. Targeting a selected panel of host factors mostly involved in the latter in ACHN cells by RNAi, we detected that silencing of distinct targets followed by Stx exposure yielded a substantial decrease in toxin-mediated cytotoxicity. These included the well-established YKT6, showing the validity of our approach, but also in Stx pathobiology totally unprecedented RAB5A and TRAPPC6B. Moreover, combining the knockdowns of these targets nearly completely abolished the Stx-mediated cytotoxicity.

## Conclusions

New host factors involved in the retrorouting of Stx, thus representing potential targets for the inhibition of Stx-caused cellular injury, were identified by a combined approach of comparative transcriptomics and RNAi. Our study therefore may support the search for novel targets as therapeutic options to combat devastating EHEC infections.

## 183-ZOV

**Transcriptome analysis reveals profound effect of Shiga toxin phage carriage on *E. coli* O104:H4 motility and virulence gene expression**

\*P. Berger<sup>1</sup>, I. U. Kouzel<sup>2</sup>, M. Berger<sup>1</sup>, N. Haarmann<sup>3</sup>, H. Karch<sup>1</sup>, A. Mellmann<sup>1</sup>

<sup>1</sup>University of Münster, Institute of Hygiene, Münster, Germany

<sup>2</sup>University of Bergen, Sars International Centre for Marine Molecular Biology, Bergen, Germany

<sup>3</sup>University of Hohenheim, Institute of Food Science and Biotechnology, Stuttgart, Germany

## Question

Shiga toxins (Stx) phages can lysogenize susceptible bacteria and thereby either transform commensal strains into pathogens, or increase the virulence of already pathogenic bacterial hosts. Moreover, there is evidence that Stx phage-encoded factors modulate bacterial host gene expression. We recently showed that Stx2 phage carriage has a profound impact on the transcriptome and phenotype of the commensal *E. coli* K-12 MG1655. Here, we investigate the Stx2 phage-dependent effects on the transcriptome of the highly virulent *E. coli* O104:H4 outbreak strain.

## Methods

RNA-seq was performed with total RNA from wild type and Stx2 phage cured *E. coli* O104:H4. Directional cDNA libraries were sequenced on Illumina platform. Raw data were processed using READemption and differences in gene expression were determined by DESeq2. Protein levels were analysed with semi-quantitative Western blot. The swimming motility was monitored on LB plates with 0.3 % agar.

## Results

On average 11 million reads were sequenced per library and at least 96% of them could be mapped to the reference genome of *E. coli* O104:H4. DESeq2 analysis revealed 466 upregulated and 356 downregulated genes in the wild type strain in comparison to its Stx2 phage-cured derivative. The most populated group of upregulated genes in the wild type *E. coli* O104:H4 were genes involved in motility. FliC western blot analysis and motility assays confirmed that these changes in the gene expression resulted in stable phenotypic changes. In addition, the Stx2 phage carriage led to the upregulation of both chromosomal and pAA plasmid-encoded known virulence-associated genes, e.g. genes encoding the serine proteases Pic, SigA and SepA, as well as genes mediating the aggregative adherence of the outbreak strain (*aggR*, *aggD-A*).

## Conclusions

Our study reveals a profound impact of the Stx2 phage carriage on *E. coli* O104:H4 gene expression. We confirm the enhanced expression of flagella, which may stimulate the inflammatory response in the human host. In addition, we detect an extensive crosstalk between the Stx2 phage carriage and the expression of chromosome and pAA-encoded virulence genes.

## 184-ZOV

Role of phage-encoded NanS-p O-acetyl esterases of pathogenic *E. coli* O157:H7 and O104:H4 strains in mucin degradation and adherence to epithelial cell lines

\*H. Schmidt<sup>1</sup>, N. Saile<sup>1</sup>, S. Feuerbaum<sup>1</sup>, G. Pohlentz<sup>2</sup>, J. Muthing<sup>2</sup>, N. Haarmann<sup>1</sup>

<sup>1</sup>Institute of Food Science and Biotechnology, University of Hohenheim, Food Microbiology and Hygiene, Stuttgart, Germany

<sup>2</sup>University of Münster, Institute of Hygiene, Münster, Germany

**Introduction.** The Shiga toxin genes of enterohemorrhagic *Escherichia coli* (EHEC) are located in distinct positions close to the antiterminator Q in the late transcribed region of prophages. Further non-Stx-encoding lambdoid prophages are present in varying numbers in the chromosome of EHEC strains. In a number of EHEC strains, the recently described *nanS-p* genes occur in multiple copies per strain in 3'-direction close to the *stx* genes and in non-Stx-phages. The *nanS-p* genes are homologous to the chromosomal *nanS* gene, the latter of which is present in most *E. coli* strains. NanS is an esterase, which is able to cleave an acetyl residue from 5-N-acetyl-9-O-acetyl neuraminic acid (Neu5,9Ac2), a sialic acid derivative occurring in intestinal mucus. The resulting Neu5Ac can be used as a carbon source.

**Objectives.** In the current study, the role of multiple *nanS-p* genes and their gene products for substrate utilization and adherence to epithelial cells was investigated.

**Materials & Methods.** Deletion mutants were constructed by homologous recombination. NanS-p enzymes were recombinantly expressed as His-tagged proteins. Degradation of bovine submaxillary gland mucin (BSM) was analyzed by nanoESI MS. Cell culture experiments were carried out with LS180 and HT-29-cells.

**Results.** Recombinant NanS-p proteins were shown to function as O-acetyl esterases. *E. coli* O157:H7 strain EDL933 grew well on Neu5,9Ac2 as a carbon source, but mutants with deletion of all seven *nanS-p* alleles did not. *E. coli* O104:H4 strain C227-11φcu harbors four *nanS-p* genes and we could show that it can outcompete commensal *E. coli*, when Neu5,9Ac2 was used as carbon source.

Moreover, analysis of BSM degradation by recombinant NanS-p enzymes using nanoESI MS demonstrated cleavage of up to three acetyl residues from sialic acids. In addition, we could show that NanS-p also influences the adherence of EDL933 and C227-11φcu to epithelial cells.

**Conclusions.** The results of our study let us hypothesize that multiple prophage-located *nanS-p* genes represent a mobile gene pool of EHEC, improving substrate utilization in the intestinal mucus as well as cell adherence and colonization in the large intestine.

## 185-ZOV

Effect of global regulators on pathogenicity of locus of enterocyte effacement-negative Shiga toxin-producing *E. coli*

\*L. Heinisch<sup>1</sup>, M. Krause<sup>1</sup>, H. Schmidt<sup>1</sup>

<sup>1</sup>Institute of Food Science and Biotechnology, University of Hohenheim, Food Microbiology and Hygiene, Stuttgart, Germany

**Introduction:** Shiga toxin-producing *Escherichia coli* (STEC) are a major cause of foodborne outbreaks worldwide. The best described virulence factors so far, are the Shiga toxins and the locus of enterocyte effacement (LEE), which is needed for the adhesion to host cells. In recent years, LEE-negative STEC have been associated with human diseases. Those STEC strains harbour different virulence factors, for example the subtilase cytotoxin (SubAB). Despite the knowledge of mode of action of this toxin, the regulation of the gene expression is still unknown.

**Objective:** The aim of this study was to address this issue focusing on the impact of the global regulatory proteins Hfq and H-NS on virulence gene regulation, especially on *subAB* expression. Studies were conducted with the foodborne STEC O113:H21 strain TS18/08 and the overall impact of regulatory proteins on pathogenicity of this strain was investigated.

**Material and Methods:** Isogenic deletion mutants TS18/08 Δ*hfq* and TS18/08 Δ*hns* were generated by homologous recombination. The deletion mutants were analysed according to impact on gene expression of virulence factors (quantitative real-time PCR) and on effect during adhesion and infection (cell culture experiments).

**Results:** Upregulation of *stx*, *subAB*, and of the cytolethal distending toxin V gene (*cdt-V*) were observed in deletion mutants TS18/08 Δ*hfq* and TS18/08 Δ*hns*. Moreover, deletion of genes *hfq* and *hns* led to an altered pathogenicity during adhesion assays.

**Conclusions:** These data reveal that toxins and factors for adhesion and pathogenicity of those STEC strains are integrated in the regulatory circuit of Hfq and H-NS. Moreover, regulation of virulence factors might be integrated in the bacterial growth cycle and therefore pathogenicity of LEE-negative STEC strains should be investigated in more detail.

## 186-ZOV

Whole genome sequencing reveals extended natural transformation in *Campylobacter* impacting diagnostics and the pathogens adaptive potential

J. Golz<sup>1</sup>, L. Epping<sup>2</sup>, M. T. Knüver<sup>1</sup>, M. Borowiak<sup>1</sup>, F. Hartkopf<sup>2</sup>, C. Deneke<sup>1</sup>, B. Malorny<sup>1</sup>, T. Semmler<sup>2</sup>, \*K. Stingl<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Berlin, Germany

<sup>2</sup>Robert Koch Institute, Berlin, Germany

## Introduction:

*Campylobacter* is the major bacterial agent of human gastroenteritis worldwide and represents a crucial global public health burden. Species differentiation of *C. jejuni* and *C. coli* and phylogenetic analysis is challenged by inter-species horizontal gene transfer.

## Objectives:

To characterize *Campylobacter* isolates with ambiguous species differentiation results obtained by real-time PCR, we performed whole genome sequencing and further characterized the isolates.

## Method:

Routine real-time PCR was performed on more than 4000 isolates obtained from the Federal state laboratories. The isolates with ambiguous PCR results were examined by whole-genome sequencing and data were analysed using Ridom Seqsphere+ software, web-based analysis and in-house k-mer analysis pipeline.

## Results:

Isolates with ambiguous PCR results for species differentiation were, in particular, identified from the isolation source eggs. K-mer analysis of whole genome sequencing data indicated the presence of *C. coli* hybrid strains with huge amounts of *C. jejuni* introgression. Recombination events were distributed over the whole chromosome. MLST was not suitable, since *C. jejuni* sequences were also found in six of the seven housekeeping genes. cgMLST suggested that the strains were phylogenetically unrelated. Intriguingly, the strains shared a stress response set of *C. jejuni* variant genes, with proposed roles in oxidative, osmotic and general stress defence, chromosome maintenance and repair, membrane transport, cell wall and capsular biosynthesis and chemotaxis.

## Conclusion:

Natural transformation can lead to immense interspecies gene transfer in *C. coli*. The results have practical impact on routine typing and on the understanding of the functional adaptation to harsh environments, enabling successful spreading and persistence of thermophilic *Campylobacter*.

## 187-ZOV

Carvacrol ameliorates acute campylobacteriosis in a clinical murine infection model

\*S. Bereswill<sup>1</sup>, S. Mousavi<sup>1</sup>, U. Escher<sup>1</sup>, A. M. Schmidt<sup>1</sup>, S. Kittler<sup>2</sup>, C. Kehrenberg<sup>3</sup>, E. Thunhorst<sup>2</sup>, M. M. Heimesaat<sup>1</sup>

<sup>1</sup>Charité - University Medicine Berlin, Pharmaceutical Microbiology Group/ Microbiology, Infectious Diseases and Immunology, Berlin, Germany

<sup>2</sup>University of Veterinary Medicine, Institute for Food Quality and Food Safety, Hannover, Germany

<sup>3</sup>Justus-Liebig-University, Institute for Veterinary Food Science, Giessen, Germany

The prevalence of human infections with the zoonotic pathogen *Campylobacter jejuni* is rising worldwide. Therefore, the identification of compounds with potent anti-pathogenic and anti-inflammatory properties for future therapeutic and/or preventive application to combat campylobacteriosis is of importance for global health. Results of recent studies suggested carvacrol (4-isopropyl-2-

methylphenol) as potential candidate molecule for the treatment of campylobacteriosis in humans and for the prevention of *Campylobacter* colonization in farm animals.

To address this in a clinical murine infection model of acute campylobacteriosis, secondary abiotic IL-10-/- mice were subjected to synthetic carvacrol via the drinking water starting four days before peroral *C. jejuni* challenge. Whereas at day 6 post-infection placebo treated mice suffered from acute enterocolitis, mice from the carvacrol cohort not only harbored two log orders of magnitude lower pathogen loads in their intestines, but also displayed significantly reduced disease symptoms. Alleviated campylobacteriosis following carvacrol application was accompanied by less distinct intestinal apoptosis and pro-inflammatory immune responses as well as by higher numbers of proliferating colonic epithelial cells. Remarkably, the inflammation-ameliorating effects of carvacrol treatment were not restricted to the intestinal tract, but could also be observed in extra-intestinal organs such as liver, kidneys and lungs and, strikingly, systemically as indicated by lower IFN- $\gamma$ , TNF, MCP-1 and IL-6 serum concentrations in carvacrol versus placebo treated mice. Furthermore, carvacrol treatment was associated with less frequent translocation of viable *C. jejuni* originating from the intestines to extra-intestinal compartments.

The lowered *C. jejuni* loads and alleviated symptoms observed in the here applied clinical murine model for human campylobacteriosis highlight the application of carvacrol as a promising novel option for both, the treatment of campylobacteriosis and hence, for prevention of post-infectious sequelae in humans, and for the reduction of *C. jejuni* colonization in the intestines of vertebrate livestock animals.

## 188-ZOV

Impact of milk and peptide utilization on the metabolic fitness and proliferation of *Brucella melitensis* and *Brucella abortus*

A. Stephan<sup>1</sup>, S. Al Dahouk<sup>1</sup>, \*D. Hofreuter<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment (BfR), Department of Biological Safety, Berlin, Germany

Brucellosis is a widespread zoonotic disease primarily transmitted by the consumption of unpasteurized dairy products contaminated with *Brucella abortus* or *Brucella melitensis*. Both *Brucella* species are viable in milk for weeks, but our knowledge about the impact of milk on the metabolic activity and propagation of *Brucella* is limited. We therefore examined, to what extent both *Brucella* species are able to use milk as growth substrate.

Culture experiments demonstrated that *B. abortus* and *B. melitensis* proliferate efficiently in commercial cow's milk reaching high numbers with a 10<sup>5</sup>-fold increase after incubating 10<sup>3</sup> bacteria for 3 days. To examine the role of milk peptides for the observed growth, we cultivated both *Brucella* species in medium with tryptic-digested casein or casein hydrolysate as sole energy sources. These experiments revealed that casein peptides efficiently facilitated *Brucella* growth. Comparative genome analyses of both *Brucella* species identified several proteases, peptidases and peptide transporters that could support the utilization of milk proteins. In addition, Micronaut<sup>TM</sup> analyses including various chromogenic peptidase substrates identified pronounced aminopeptidase activities that differ significantly between *Brucella* species. To characterize the substrate specificity of the *Brucella* peptidases we performed a comprehensive screening with over 250 dipeptides using

the BIOLOG™ phenotypic microarray assay. Several dipeptides are utilized by *B. abortus* and *B. melitensis*, but we also identified striking differences in the dipeptide utilization pattern of the two *Brucella* species. These results suggest distinct peptide catabolism patterns in *Brucella* species that might reflect adaptations to their different hosts or nutritional niches. Ongoing comparative analyses aim to identify the genomic variabilities that shape these altered phenotypes.

The ability of *Brucella* to grow in milk suggests a higher risk of exposure when refrigeration infrastructures for raw milk are insufficient, subsequently increasing the threat of *Brucella* spp. as select agents in case of deliberate releases of low numbers of bacteria during dairy production.

---

### 189-ZOV

Functional analysis of *ica*-like genes in livestock-associated *Staphylococcus sciuri* isolates

G. Marincola<sup>1</sup>, G. Jaschkowitz<sup>1</sup>, A. K. Kieninger<sup>1</sup>, A. Feßler<sup>2</sup>, S. Schwarz<sup>2</sup>, \*W. Ziebuhr<sup>1</sup>

<sup>1</sup>University of Würzburg, Molecular Infection Biology, Würzburg, Germany

<sup>2</sup>Freie Universität Berlin, Institut für Mikrobiologie und Tierseuchen, Berlin, Germany

Biofilm formation is a common feature of staphylococci conferring protection against unfavorable environmental conditions, and contributing (in pathogenic species) to treatment recalcitrance and chronic infections. Biofilms are surface-attached bacterial communities surrounded by a self-produced extracellular matrix consisting of polysaccharides and/or proteins. In staphylococci, the main polysaccharide biofilm component is Polysaccharide InterCellular Adhesin (PIA). PIA is produced by the gene products of the *icaADBC* operon which are controlled by the adjacent repressor IcaR. We have previously characterized a multiresistance plasmid isolated from livestock-associated methicillin-resistant *Staphylococcus aureus* strain Rd11. Unexpectedly, this plasmid (pAFS11) harboured, in addition to resistance genes, a novel *ica*-like gene cluster. Bioinformatic analysis demonstrated that the pAFS11 *ica* genes originated from the animal-associated species *Staphylococcus sciuri* and had been acquired by *S. aureus* Rd11 via horizontal gene transfer. We have tested livestock-associated *S. sciuri* isolates for *ica* gene presence and function and found that 75 % of 158 *S. sciuri* isolates carried the *ica* gene cluster. However, none of the isolates formed a PIA-mediated biofilm. No PIA biofilm was detected in *S. aureus* Rd11, carrying the *S. sciuri* *ica* genes on plasmid pAFS11. Interestingly, like all *S. aureus* strains, Rd11 harbours its own chromosomally encoded *ica* gene cluster, and transformation of pAFS11 into a PIA-producing *S. aureus* strain resulted in reduced PIA production. Our experiments suggest a regulatory crosstalk between plasmid-encoded *S. sciuri* IcaR with the chromosomal *S. aureus* *icaADBC* operon. The data highlight ongoing genetic exchange across staphylococcal species borders which, in addition to resistance genes, may also comprise transfer of virulence traits. We hypothesize that co-selection of virulence factors by antibiotics and their integration into the regulatory circuits of the host bacterium might play an important, but still poorly understood role in the emergence of bacteria with novel resistance and virulence traits.

---

### 190-MIEV

Molecular characterization of pneumococcal isolates from children vaccinated with PCV-13 in Cape Coast, Ghana

\*R. O. Mills<sup>1,2</sup>, M. R. Abdullah<sup>1</sup>, S. Akwetey<sup>2</sup>, D. Sappor<sup>2</sup>, J. A. B. Muñoz<sup>3</sup>, G. Gámez<sup>3</sup>, M. P. G. van der Linden<sup>1</sup>, S. Hammerschmidt<sup>1</sup>

<sup>1</sup>Interfaculty Institute of Genetics and Functional Genomics Center for Functional Genomics of Microbes Universität Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

<sup>2</sup>University of Cape Coast, Biomedical Sciences, Cape Coast, Ghana

<sup>3</sup>University of Antioquia, School of Microbiology, Medellín, Colombia

**Introduction:** Globally, about 300,000 pneumococcal deaths occurred among HIV uninfected children less than five years in 2015 with the majority occurring in Africa and Asia. Studying pneumococcal carriage in the nasopharynx is one of the methods used to assess the impact of pneumococcal conjugate vaccines and monitor circulating serotypes. We investigated virulence factors, serotype distribution and antibiotic susceptibility patterns of pneumococcal carriage strains isolated from vaccinated children less than five years of age in Ghana.

**Method:** In 2018, nasopharyngeal swabs were collected from 513 fully vaccinated children attending immunization clinics and kindergartens in Cape Coast, Ghana. Standard microbiology techniques were used to identify and confirm pneumococcal isolates. Core genome virulence factors and flexible genomic regions were identified by PCR. Pneumococcal serotypes were determined by multiplexPCR and Quellung reaction. Antibiotic susceptibility was tested by the disc diffusion method.

**Results:** Our surveillance showed a pneumococcal carriage prevalence of 29.4% (151/513). Out of the twenty-six different serotypes identified, serotypes 23B, 6B, 23F and 19F (14.4%, 9.2%, 8.5% and 7.2%) were most prevalent. The proportion of serotypes present in PCV-13 was 39.1% (59/151). Major non PCV-13 serotypes were 23B and 13 (14.4% and 7.2%). All pneumococcal strains were positive for *lytA*, *cps2A* and *pavB* genes. In addition *psrP*, *pcpA*, *pilus* *PI-1* and *PI-2* were present in 62.7%, 87.5%, 11.8%, and 6.5%, of the strains respectively. The isolates were highly susceptible to levofloxacin, ceftriaxone, vancomycin and erythromycin. However, we observed increased non-susceptibility to penicillin, cotrimoxazole, and tetracycline. About 52% (79/151) of the isolates were oxacillin non-susceptible; 54 of them showed intermediate resistance to penicillin and one strain was fully resistant.

**Conclusion:** Pneumococcal carriage prevalence was 29.4%. However, there is an increase in non-PCV 13 serotypes, with serotype 23B being the most dominant, with associated high penicillin resistance. Effective regulation on appropriate use of antibiotics is therefore of essence in Ghana.

---

### 191-MIEV

**ASA<sup>3</sup>P: An automatic and scalable pipeline for the assembly, annotation and higher level analysis of closely related bacterial isolates**

\*O. Schwengers<sup>1,2</sup>, A. Hoek<sup>1</sup>, M. Fritzenwanker<sup>2</sup>, L. Falgenhauer<sup>2</sup>, T. Hain<sup>2</sup>, T. Chakraborty<sup>2</sup>, A. Goesmann<sup>1</sup>

<sup>1</sup>Justus-Liebig-University Giessen, Bioinformatics & Systems Biology, Giessen, Germany

<sup>2</sup>Justus-Liebig-University Giessen, Institute of Medical Microbiology, Giessen, Germany

### Question

Major technological advances and the dramatic decrease in costs of bacterial whole genome sequencing is having an unprecedented effect in microbial epidemiology. These developments require the establishment of effective, efficient

and scalable bioinformatics software tools for raw processing and analysis of the high-throughput data before scientific interpretation can take place.

## Methods

In order to solve core bioinformatics tasks such as quality trimming, assembly and annotation, ASA<sup>3</sup>P takes advantage of published and well performing third party tools and combines them with comprehensive databases. It is a modular and extensible software pipeline implemented in Java and Groovy. For massive scalability our pipeline takes advantage of SGE compatible compute clusters.

## Results

Here, we introduce ASA<sup>3</sup>P, a fully automatic and scalable assembly, annotation and higher-level analysis pipeline for bacterial genomes. The pipeline conducts all of the necessary data processing steps, *i.e.* quality clipping and assembly of sequencing reads, scaffolding subsequent contigs and annotation of genome sequences. Furthermore, ASA<sup>3</sup>P performs comprehensive genome characterizations and analyses, *e.g.* for taxonomic classification, and detection of both AMR genes and virulence factors. Results are presented via an HTML5 based user interface providing aggregated information, interactive visualizations and access to intermediate results in standard bioinformatic file formats. ASA<sup>3</sup>P is available in two versions: a local Docker container for small-scale projects and an OpenStack cloud version able to automatically create and manage its own self-scaling compute cluster.

## Conclusions

ASA<sup>3</sup>P is a software tool enabling the automatic processing, assembly, annotation and higher level analysis of bacterial NGS whole genome data in a comfortable but high-throughput manner. The burden of technical complexity is overcome by simple setup routines and the use of Docker and OpenStack images. Thus, automatic and standardized analysis of hundreds of bacterial genomes is now feasible on a daily basis with a single command on local computers as well as cloud infrastructures.

### 192-MIEV

Genome-wide identification of Host-Specificity of *Campylobacter jejuni* in Germany based on Whole Genome Data

\*L. Epping<sup>1</sup>, R. M. Piro<sup>2,1</sup>, M. T. Knüver<sup>3</sup>, M. Borowiak<sup>4</sup>, C. Huber<sup>5</sup>, A. Thürmer<sup>6</sup>, B. Malorny<sup>4</sup>, K. Stingl<sup>3</sup>, A. Fruth<sup>7</sup>, L. H. Wieler<sup>6</sup>, T. Semmler<sup>8</sup>

<sup>1</sup>Charité Universitätsmedizin, Institute of Medical Genetics and Human Genetics, Berlin, Germany

<sup>2</sup>Freie Universität, Mathematics and Computer Science, Berlin, Germany

<sup>3</sup>German Federal Institute for Risk Assessment, National Reference Laboratory for *Campylobacter*, Berlin, Germany

<sup>4</sup>German Federal Institute for Risk Assessment, Study Centre for Genome Sequencing and Analysis, Berlin, Germany

<sup>5</sup>Robert Koch Institute, Advanced Light and Electron Microscopy, Berlin, Germany

<sup>6</sup>Robert Koch Institute, Methodology and Research Infrastructure, Berlin, Germany

<sup>7</sup>Robert Koch Institute, National Reference Centre for *Salmonella* and other Bacterial Enterics, Berlin, Germany

<sup>8</sup>Robert Koch Institute, Microbial Genomics, Berlin, Germany

**Introduction** During the last two decades the zoonotic pathogen *Campylobacter jejuni* has become the main cause for food-borne infections in high-income countries.

Colonization and adaptation of multiple host reservoirs leads to a tremendous risk of contamination in food products. Most commonly *Campylobacter* are transmitted through the consumption of undercooked poultry meat or raw milk products. While host restriction of *C. jejuni* lineages is known, the survival mechanisms of them to adapt to gut environments of different hosts have not been completely understood.

**Objectives** This study was designed to support outbreak investigations against the threat of increasing numbers of *C. jejuni* infections in humans. Its main aim is to gain additional insights into the *C. jejuni* population structure in Germany and to identify host specific determinants by using novel computational methods based on genomic data.

**Material & methods** Therefore, 330 *C. jejuni* strains from different hosts (100 each from human, chicken, cattle and 30 from pig) across Germany were randomly selected, and whole genome sequencing (WGS) was performed. To extend the dataset and compare it with international samples, 166 additional isolates from a Canadian study were included. Host-specificity was investigated by a stratified random sampling approach on top of a *k-mer* based genome-wide association study to increase the accuracy of the identification of host specific determinates.

**Results** In our study, we discovered that a strong host association can be observed in the core genome as well as in the accessory genome. The identified genetic elements code for proteins, which play important roles in mobility, energy metabolism and genetic information processing. Although, we could discover a strong recombination barrier between *C. jejuni* lineages within the same host, identical allelic gene variants could be found among those genes.

**Conclusion** Host-adaptation in a wide range of cellular functions in the whole pan-genome of *C. jejuni* indicates that the adaptation towards a specific host niche is most likely a long evolutionary and multifactorial process rather than a spontaneous evolution or selection pressure.

### 193-MIEV

Surveillance of *Salmonella Agona* outbreaks in Bavaria by Next Generation Sequencing

\*A. Dangel<sup>1</sup>, A. Berger<sup>1</sup>, U. Messelhäuser<sup>1</sup>, R. Konrad<sup>1</sup>, S. Hörmansdorfer<sup>1</sup>, A. Sing<sup>1</sup>

<sup>1</sup>Bavarian Health and Food Safety Authority (LGL), Oberschleißheim, Germany

*S. Agona* is one of the *Salmonella enterica* subsp. *enterica* serovars consistently responsible for foodborne outbreaks via various food types all over the world. In 2017, an outbreak with nearly 40 patients based on infant milk products of a French supplier occurred in several European countries. At the same time, *S. Agona* was detected in animal feed samples in Bavaria and a connection of both events was at question.

The aim of this study was to evaluate different data analyses approaches and necessary analysis depth for Next Generation Sequencing (NGS)-based *S. Agona* surveillance and outbreak detection by public health authorities. Therefore, 51 Bavarian isolates from 1994 to 2018 from food, feed and human patients, including known former outbreak isolates and the Bavarian feed isolates of 2017 were used as evaluation sample set together with a public NGS dataset of a representative isolate of the 2017 French infant milk outbreak.



NGS analysis was performed on the Illumina MiSeq. Three data analysis approaches for public health purposes were compared: core genome Multi Locus Sequence Typing (cgMLST) with two different target schemes or Single Nucleotide Polymorphism (SNP) phylogeny.

With the NGS analysis we could prove clonality of the Bavarian feed isolates and exclude any connection to the simultaneous French outbreak. The molecular approach furthermore revealed and confirmed former Bavarian clusters with known epidemiological links. The necessary genetic resolution regarding analysed genomic content and consequential cluster demarcation was evaluated by comparison of a publicly available species-specific cgMLST scheme, an in-house serovar *Agona*-specific cgMLST scheme and SNP phylogeny.

In conclusion, even for the genetically monomorphic serovar *S. Agona*, species level cgMLST can usually serve as fast and easy applicable analysis technique with reasonable resolution, being standardisable for a wide field of applications for public health laboratories. Nevertheless, in special cases of single samples or homogeneous sample sets higher resolution by serovar-specific cgMLST or SNP genotyping can facilitate the assignment to or delineation from an outbreak.

#### 194-MIEV

Penicillin binding proteins - the one and only key to  $\beta$ -lactam resistance in *Staphylococcus aureus*?

\*A. Sommer<sup>1</sup>, M. B. Erdmann<sup>1</sup>, S. Fuchs<sup>1</sup>, G. Werner<sup>1</sup>, B. Strommenger<sup>1</sup>

<sup>1</sup>Robert Koch-Institute, Department of Infectious Diseases, Wernigerode, Germany

**Question:** In *Staphylococcus aureus* resistance to  $\beta$ -lactamase stable  $\beta$ -lactam antibiotics, like methicillin, is commonly mediated by the *mecA*-encoded penicillin binding protein 2a (PBP2a) or the *mecC*-encoded PBP2c. These cell wall-associated proteins exhibit low affinity to most  $\beta$ -lactam antibiotics. The number of isolates showing methicillin resistance while lacking known *mec* genes (MRLM) sent to the German National Reference Center for Staphylococci and Enterococci increased over the past years. Therefore the present study aims to investigate the resistance mechanisms of these isolates using a combination of bioinformatics and molecular approaches. **Methods:** Minimal inhibitory concentrations to oxacillin and cefoxitin of clinical *S. aureus* isolates were determined by applying broth microdilution. The presence of *mecA* and *mecC* was tested via PCR. Hyperproduction of  $\beta$ -lactamase leading to borderline resistance phenotype (BORSA) was excluded. A strain collection of MRLM isolates for the implementation of genome-wide association studies (GWAS) was established. MRLM isolates and methicillin susceptible controls (MSSA) were whole-genome sequenced and subsequently characterized by *spa* typing, MLST and core genome MLST (cgMLST) analysis. **Results:** In total 141 isolates were determined as resistant to oxacillin and cefoxitin, while lacking *mecA* and *mecC* genes. The sequenced MRLM isolates were assigned to 29 sequence types (ST) and 73 *spa* types. Overall the established MRLM strain collection, including 142 MSSA controls, covers a wide range of clonal complexes, with cases and controls evenly distributed. **Conclusions:** GWAS will be performed to determine genetic variants associated to  $\beta$ -lactam resistance. Furthermore, the impact of putative resistance associated polymorphisms has

to be confirmed by construction and validation of corresponding mutants.

#### 195-MIEV

**Molecular analysis of a *Serratia marcescens* colonisation outbreak in a neonatal intensive care unit indicates a role for the type 6 secretion system**

M. Werner<sup>1</sup>, A. Ensser<sup>1</sup>, G. Valenza<sup>2</sup>, F. Fahlbusch<sup>1</sup>, S. Coulthurst<sup>3</sup>, B. Kunz<sup>1</sup>, C. Bogdan<sup>1</sup>, \*R. Lang<sup>1</sup>

<sup>1</sup>University Hospital Erlangen, Erlangen, Germany

<sup>2</sup>Landesamt für Gesundheit und Lebensmittelsicherheit, Erlangen, Germany

<sup>3</sup>University of Dundee, Dundee, United Kingdom

The microbial spectrum of infection outbreaks in neonatal intensive care units (NICU) shows a high prevalence of enterobacteria, especially of *Serratia* (*S.*) *marcescens* that accounts for 12% of all outbreaks. To date, it is unknown why *S. marcescens* is so successful in colonization and infection of newborns.

Here, we report a colonization outbreak with *S. marcescens* in a university hospital NICU. Following detection of *S. marcescens* in clinical samples of three patients, prevalence screening by nasopharyngeal and anorectal swabs identified additional 11 carriers. The outbreak management team devised a bundle of measures (incl. cohort isolation of colonized/contact patients; admission and interval screening of patients; training of staff and parents by hygiene specialists; screening of environmental samples and personnel). Cumulatively, a total of 30 children were found to be colonized until the end of the outbreak 6 weeks later.

All *S. marcescens* isolates were analyzed for clonality by PCR-based DiversiLab-typing and by Pulsed Field Gel Electrophoresis (PFGE), revealing that 24/30 patients carried the outbreak isolate, whereas the others harbored unrelated strains. While isolates from personnel were unrelated, the outbreak isolate was found in two environmental samples from baby bottle warmers. Whole Genome Sequencing (WGS) via Illumina MiSeq confirmed the PFGE-typing results and revealed identical sequences for the outbreak isolates from patients and vaporizer.

Recently, a *S. marcescens* type 6 secretion system (T6SS) for injection of effector proteins was identified as effective nano-weapon for interbacterial competition. Indeed, the outbreak isolates analyzed by MiSeq harbored a complete T6SS locus, whereas several sporadic *S. marcescens* isolates lacked specific effector genes. Bacterial competition assays revealed efficient killing of *E. coli* by the outbreak isolates, whereas *S. marcescens* lacking different effector genes displayed reduced killing capacity. These results indicate that the complete T6SS nano-machinery may provide the outbreak strain with a competitive advantage for colonization of neonates.

#### 196-MIEV

A publicly accessible database for *Clostridioides difficile* genome sequences supports tracing of transmission chains and epidemics

\*M. Frentrop<sup>1</sup>, Z. Zhou<sup>2</sup>, M. Steglich<sup>1,3</sup>, J. Meier-Kolthoff<sup>1</sup>, M. Göker<sup>1</sup>, T. Riedel<sup>1,3</sup>, B. Bunk<sup>1</sup>, C. Spröer<sup>1</sup>, J. Overmann<sup>1,3,4</sup>, M. Blaschitz<sup>5</sup>, A. Indra<sup>5</sup>, L. von Müller<sup>6</sup>, T. A. Kohl<sup>7,8</sup>, S. Niemann<sup>7,8</sup>, C. Seyboldt<sup>9</sup>, F. Klawonn<sup>10,11</sup>, N. Kumar<sup>12</sup>, T. D. Lawley<sup>12</sup>, S. García-Fernández<sup>13,14</sup>, R. Cantón<sup>13,14</sup>, R. del Campo<sup>13,14</sup>, O. Zimmermann<sup>15</sup>, U. Groß<sup>15</sup>, M. Achtman<sup>2</sup>, U. Nübel<sup>1,3,4</sup>

<sup>1</sup>Leibniz-Institute DSMZ, Braunschweig, Germany

<sup>2</sup>Warwick Medical School, University of Warwick, Warwick, United

Kingdom

<sup>3</sup>German Center for Infection Research (DZIF), Braunschweig, Germany

<sup>4</sup>Integrated Center of Systems Biology (BRICS), Braunschweig, Germany

<sup>5</sup>AGES - Austrian Agency for Health and Food Safety, Wien, Austria

<sup>6</sup>Christophorus-Kliniken, Coesfeld, Germany

<sup>7</sup>Research Center Borstel, Borstel, Germany

<sup>8</sup>German Center for Infection Research (DZIF), Borstel, Germany

<sup>9</sup>Friedrich-Loeffler-Institut, Jena, Germany

<sup>10</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

<sup>11</sup>Institute for Information Engineering, Ostfalia University, Wolfenbüttel, Germany

<sup>12</sup>Wellcome Sanger Institute, Hinxton, United Kingdom

<sup>13</sup>Servicio de Microbiología, Hospital Universitario Ramón y Cajal, and Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

<sup>14</sup>Red Española de Investigación en Patología Infecciosa (REIPI), Madrid, Spain

<sup>15</sup>University Medical Center, Göttingen, Germany

*Clostridioides difficile* is the primary infectious cause of antibiotic-associated diarrhea. Local transmissions and international outbreaks of this pathogen have been previously elucidated by bacterial whole-genome sequencing, but comparative genomic analyses at the global scale were hampered by the lack of specific bioinformatic tools. We have implemented a publicly accessible database within Enterobase (<http://enterobase.warwick.ac.uk>) that automatically retrieves and assembles *C. difficile* short-reads from the public domain, and calls alleles for core-genome multilocus sequence typing (cgMLST). Enterobase currently contains 13,515 quality-controlled *C. difficile* genomes which have been assigned to hierarchical sets of single-linkage clusters by cgMLST distances. This hierarchical clustering is used to identify and name populations of *C. difficile* at all epidemiological levels, from recent transmission chains through to pandemic and endemic strains. Moreover, it puts newly collected isolates into phylogenetic and epidemiological context by identifying related strains among all previously published genome data. For example, HC2 clusters (i.e. chains of genomes with pairwise distances of up to two cgMLST alleles) were statistically associated with specific hospitals ( $p < 10^{-4}$ ) or single wards ( $p = 0.01$ ) within hospitals, indicating they represented local transmission clusters. In contrast, clustering at level HC150 was largely compatible with PCR ribotyping, thus enabling comparisons to earlier surveillance data. Enterobase enables contextual interpretation of a growing collection of assembled, quality-controlled *C. difficile* genome sequences and their associated metadata. Hierarchical clustering rapidly identifies database entries that are related at multiple levels of genetic distance, facilitating communication among researchers, clinicians and public-health officials who are combatting disease caused by *C. difficile*.

## 197-MIEV

Molecular epidemiology of Vancomycin-resistant Enterococci: four years of state-wide surveillance in North Rhine-Westphalia

\*C. L. Correa-Martinez<sup>1</sup>, A. Jurke<sup>2</sup>, J. Schmitz<sup>1</sup>, S. Kampmeier<sup>1</sup>, A. Mellmann<sup>1</sup>

<sup>1</sup>University Hospital Münster, Institute of Hygiene, Münster, Germany

<sup>2</sup>North Rhine-Westphalian Centre for Health, Department Health Promotion Health Protection, Bochum, Germany

## Introduction

Vancomycin-resistant enterococci (VRE) are increasingly important causal agents of nosocomial infections. Thus, reliable epidemiological data are needed to understand their spread dynamics and to describe the magnitude of the problem. The incorporation of typing data allows to define the

temporal and geographical distribution of VRE, which is essential to implement effective infection control strategies.

## Objectives

The aim of this ongoing study is to describe the temporal and geographical distribution of invasive VRE infections in North Rhine-Westphalia (NRW) by employing molecular typing methods.

## Materials and Methods

Bacterial isolates of all patients with invasive VRE infections (blood cultures or cerebrospinal fluid [CSF]) were collected between 2016 and 2019. Inclusion criteria were place of residence and/or hospitalisation in NRW. For genotypic characterisation, multilocus sequence typing (MLST) and vancomycin resistance determinants were analysed.

## Results

Preliminary results of 717 VRE isolates detected in blood cultures or CSF show a steady increase of annual infections: 93, 156 and 244 isolates in 2016, 2017, and 2018, respectively, as well as 224 as of October 2019. Incidence was highest among men (61%), and patients aged 70-79 (30.8%). Overall, the most prevalent MLST sequence types (ST) were ST117 (68.6%), ST80 (15.5%) and ST203 (5.3%), observing a sustained increase of ST117 and the emergence of other STs (ST1273, ST1325, ST787) over the study period. In 2016, most isolates (64.5%) displayed the *vanA* genotype, however, this shifted to *vanB* in 2017 (68.59%), a distribution that continued over the following years (2018: 83.2% and 2019: 75.3%).

## Conclusion

As also reported in other countries, invasive VRE infections are on the rise in NRW. Molecular typing data indicate a growing genetic diversity among VRE isolates. However, ST117 accounts for the overall increase in the number of cases over time. *vanB* has overtaken *vanA*, historically the most prevalent resistance determinant in Germany and Europe. Our results underline the importance of infection prevention and control policies in order to reduce the regional spread of VRE.

## 198-EMV

Population-level transcriptional responses to wetting and desiccation in an arid biological soil crust

\*S. Imminger<sup>1</sup>, D. Meier<sup>1</sup>, A. Schintlmeister<sup>1,2</sup>, D. Woebken<sup>1</sup>

<sup>1</sup>University of Vienna, Centre for Microbiology and Environmental System Science, Vienna, Austria

<sup>2</sup>University of Vienna, Large-Instrument Facility for Advanced Isotope Research, Vienna, Austria

Drylands account for the largest terrestrial biome on earth and cover about 35% of the lands surface. Low water availability is one of the major factors that limit the growth of vascular plants in these ecosystems. Therefore, drylands are mainly inhabited by diverse and highly adapted microbial surface communities, forming so-called biological soil crusts (BSCs). Besides limited water availability these communities face the challenges of high UV irradiation, temperature and osmotic stress. To survive long phases of drought microorganisms enter a state of low metabolic activity, but are able to resuscitate fast to use short periods when water and nutrients become available. We investigated the response of BSC microbial communities from the Negev

Desert (Israel) to a simulated rain event covering a complete wetting/desiccation cycle. Incubations with heavy water (D<sub>2</sub>O) and subsequent high-resolution secondary ion mass spectrometry (NanoSIMS) measurements revealed a fast resuscitation of single cells in the first hours. Correspondingly, the majority of microbial populations dramatically changed their transcription profiles already within first 3 hours, based on metatranscriptome data spanning time points between 15 min and 55 hours after wetting. We observed e.g. regulation patterns for stress tolerance against reactive oxygen species, organic substrate uptake and usage of atmospheric gases as energy sources. Population-resolved analysis further revealed different expression patterns for the same metabolic pathways, e.g. chlorophyll synthesis, amongst different populations, thus highlighting microbial niche differentiation. Together with the information about the metabolic potential of the different community members, these transcriptome profiles elucidate different strategies to persist unfavorable conditions during drought and to quickly respond to water availability.

## 199-EMV

### Chemolithoautotrophic primary production sustains microbial food webs in oxic groundwater

\*M. Taubert<sup>1</sup>, G. Azemtso<sup>2,3,4</sup>, R. Houhou<sup>2,3,4</sup>, N. Jehmlich<sup>5</sup>, M. von Bergen<sup>5,6</sup>, P. Rösch<sup>2,3,4</sup>, J. Popp<sup>2,3,4</sup>, K. Küsel<sup>1,7</sup>

<sup>1</sup>Institute for Biodiversity, FSU Jena, Jena, Germany

<sup>2</sup>Institute of Physical Chemistry and Abbe Center of Photonics (IPC), FSU Jena, Jena, Germany

<sup>3</sup>Leibniz Institute of Photonic Technology (Leibniz-IPHT), Jena, Germany

<sup>4</sup>Research Campus Infectognostics e.v., Jena, Germany

<sup>5</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Molecular Systems Biology, Leipzig, Germany

<sup>6</sup>Institute of Biochemistry, University of Leipzig, Leipzig, Germany

<sup>7</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

Due to the absence of sunlight, microbial food webs in subsurface environments cannot be driven by photosynthetic primary production. Instead, hotspots of nitrogen- and sulfur-driven chemolithoautotrophy are present in pristine groundwater<sup>1</sup>. This primary production might fuel the entire groundwater microbiome, but the microbes benefiting from the organic carbon produced are unknown. Therefore, we used <sup>13</sup>CO<sub>2</sub> and D<sub>2</sub>O metabolic labeling of groundwater microcosms to track the carbon flow through the food web. Chemolithoautotrophs were stimulated with reduced sulfur compounds, and the microbial activity was monitored over 10 weeks. After 3, 6 and 10 weeks, microbial biomass was collected for DNA-based community profiling and metaproteomics analysis. Raman microspectroscopy detected deuterium incorporation in microbial cells from 12 days onwards, indicating activity of the groundwater organisms. Thiosulfate and oxygen consumption occurred within the first 3 weeks, and increased two to three-fold after 5 weeks, suggesting sulfur-driven primary production. Putative autotrophs able to oxidize sulfur compounds, like *Thiobacillus*, *Rhodoferrax* and *Hydrogenophaga*, composed up to 25 of the microbial community. Interestingly, the <sup>13</sup>C incorporation in peptides of most of these organisms ranged from 10 to 90%, indicating assimilation of unlabeled organic carbon present in the groundwater in addition to <sup>13</sup>CO<sub>2</sub>, and thus a mixotrophic lifestyle. Only *Thiobacillus* assimilated <sup>13</sup>CO<sub>2</sub> exclusively, resulting in a <sup>13</sup>C incorporation of > 95%. The majority of the community consisted of heterotrophs typical for groundwater, including various *Proteobacteria*, *Sediminibacterium* and *Flavobacterium*, as well as *Cand.* *Patescibacteria*. The <sup>13</sup>C incorporation in their peptides demonstrated assimilation of organic carbon produced by sulfur-dependent primary production. Hence, in the oligotrophic groundwater, such chemolithoautotrophic activity

might form the basis for a self-sufficient community constituting more than 50% of the groundwater microbiome.

This work was financially supported by the Deutsche Forschungsgemeinschaft via the CRC 1076 AquaDiva.

<sup>1</sup>Wegner et al. 2019, AEM, 85(5), e02346-18

## 200-EMV

### The Good, the Bad, and the Smelly – Ecology of the Human Axillary Microbiome

\*C. Koch<sup>1</sup>, S. Nordziske<sup>1</sup>, G. Schmaus<sup>1</sup>

<sup>1</sup>Symrise AG, Global Innovation Cosmetic Ingredients, Holzminden, Germany

**Introduction & Objective:** The human axillary microbiome, although being shaped by our daily hygiene routines, is far from being functionally understood. Knowing "the good, the bad, and the smelly" bacteria in the axillae is crucial for fighting malodor formation. Moreover, detailed investigations enable the targeted development of new deodorant actives without broad antimicrobial efficacy avoiding resistance development.

**Methods:** We established an *ex-vivo* human sweat model that is based on real human sweat samples and mirrors the natural microbiome development. The model allows determining microbiome functions based on chemical and sensory analyses as well as cell count (aerobic and anaerobic CFU) and microbiome composition. It was validated *in vitro* and *in vivo*.

**Results:** We were able to show the representative conversion of non-odorous precursors towards odorous products, production of malodorous volatiles from amino or fatty acid degradation, and a general increase of odor intensity based on a shift in microbiome composition and abundance. Key species for these relevant functions were identified including known malodor associated genera as well as new ones. Further, the model was used to investigate selected deodorant actives with different modes of action. One well known antimicrobial substance led to the enrichment of a resistant *Pseudomonas* strain.

**Conclusion:** The developed model represents the human axillary microbiome regarding its species composition and malodor formation. It can be used to study the microbial ecology of the axillary microbiome. Our results highlight the relevance of pre-testing new cosmetic ingredients for potential microbiome effects.

## 201-EMV

Cultivation experiments and genomic analysis suggest adaptation towards a host-independent lifestyle of a symbiont-related marine bacterium

\*K. Sass<sup>1,2</sup>, S. Güllert<sup>2</sup>, W. R. Streit<sup>2</sup>, M. Perner<sup>1</sup>

<sup>1</sup>GEOMAR Helmholtz Centre for Ocean Research Kiel, Geomicrobiology, Kiel, Germany

<sup>2</sup>Universität Hamburg, Microbiology and Biotechnology, Hamburg, Germany

Chemosynthetic bacteria living in a symbiotic relationship with invertebrate hosts like mussels or snails are widespread at hydrothermal vent habitats. These endosymbionts oxidize inorganic compounds such as hydrogen or hydrogen sulfide, emanating with the hydrothermal fluids, to gain energy. This energy can be used to fuel autotrophic CO<sub>2</sub> fixation.

Here we report on the complete genome of a newly cultured *Chromatiaceae* bacterium, namely CTD079. Its closest known relative is the endosymbiont of a scaly snail, which colonizes hydrothermal vents along the Central Indian Ridge. We enriched CTD079 from seawater of the open ocean using hydrogen and thiosulfate as sole electron donors.

Based on the close relationship between CTD079 and the endosymbiont the aim of this study was to compare their genomes in order to identify gene segments that mediate adaptation to a new habitat.

The complete genome of the newly cultivated bacterium was sequenced using Illumina MiSeq and Oxford Nanopore MinION Sequencing. Additionally, we performed *in vivo* hydrogen consumption experiments to figure out whether hydrogen could play a role for energy generation in the water column. CTD079 consumed hydrogen (0.6 fmol\* cell<sup>-1</sup> \*h<sup>-1</sup> within the first 192 hours) under autotrophic growth conditions, which suggests hydrogen as one potential energy source. Genome comparisons identified five major differences: these included gene segments that encode enzymes for the use of alternative energy sources or specific systems for the defense against viruses. These represent important attributes for a life without a host.

## 202-EMV

### The influence of precursor feeding on the production of calcimycin derivatives in *Streptomyces chartreusis*

\*K. Arend<sup>1</sup>, C. Senges<sup>1</sup>, J. Bandow<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Applied Microbiology, Bochum, Germany

The expression of secondary metabolic gene clusters in Streptomyces is highly connected to environmental conditions. The presence or absence of nutrients can either suppress or enhance secondary metabolite production. [1] Primary metabolites like amino acids, sugars, or fatty acids play an important role as building blocks for the more complex secondary metabolites. In this study we analyzed the influence of precursors on the production of the polyether ionophore calcimycin and derivatives by *Streptomyces chartreusis*. In a previous study encompassing three culture media, it was shown that their synthesis depends on the growth conditions. [2] To analyze the impact of particular precursors, we cultivated *S. chartreusis* in minimal medium supplemented with the respective precursors. Metabolites were extracted from the mycelia and culture supernatants, and analyzed by liquid chromatography-coupled tandem mass spectrometry. Calcimycin and known derivatives were identified based on their fragmentation patterns. Unknown derivatives with similar fragmentation patterns could be identified by molecular networking. [3] The production of calcimycin and derivatives was compared across growth conditions, revealing that indeed the ratio between calcimycin and the known derivatives changes depending on the presence of precursors in the medium. Based on the results of the present study, we hypothesize that *S. chartreusis* synthesizes a specific cocktail of derivatives depending on the ion and nutrient availability to promote the most efficient uptake of ions. In future studies, the novel derivatives will be purified and analyzed with regard to their structure and ion transportation capabilities to shed light on their function.

[1] A. Romero-Rodríguez *et al.*, *Antonie van Leeuwenhoek* **2018**, 111, 761.

[2] C. H. R. Senges *et al.*, *PNAS* **2018**, 115, 2490.

[3] M. Wang *et al.*, *Nat. Biotechnol.* **2016**, 34, 828.

## 203-EMV

Biological and ecological relevance of unusual sesquiterpene, sodorifen from *Serratia plymuthica* 4Rx13  
M. C. Lemfack<sup>1</sup>, P. K. Ruciaka<sup>1</sup>, \*N. Magnus<sup>1</sup>, B. Piechulla<sup>1</sup>  
<sup>1</sup>University of Rostock, Biochemistry, Rostock, Germany

Microbes produce several volatile organic compounds (mVOCs) which are compiled in a continuously updated database called "mVOC" [1]. These low molecular-weight compounds have roles in above- and below-ground interactions [2]. For many years, most focus was on above-ground roles of these VOCs. In the last two decades, there have been more studies on mVOC activities in the soils, more so the rhizosphere since *Serratia* spp. is a rich source of mVOCs [3]. *Serratia plymuthica* 4Rx13 is a producer of more than 100 volatile compounds, including the unusual VOC sodorifen [4]. In the last decade, the chemical structure of sodorifen was elucidated, and its biosynthesis and genetic regulation determined [5]. Nevertheless, the biological and ecological function(s) of this compound remains unknown.

Sodorifen in *S. plymuthica* PRI-2C increased when the bacteria were exposed to volatiles of *Fusarium culmorum* [6]. Biotic factors affect sodorifen levels ranging from inhibition to high induction levels. This correlates with the  $\sigma$ -dependent transcription of sodorifen cluster genes. Analysis of the unique gene cluster shows that fungal VOCs led to expression of methyltransferase and terpene synthase, enzymes for sodorifen synthesis (Magnus unpublished results). C-cultures of *S. plymuthica* 4Rx13 and *Bacillus subtilis* 2Bg led to altered sodorifen emission (low in exponential phase, and high in the late stationary phase) as compared to controls. Altogether, these results indicate that sodorifen plays distinct roles in nature [7].

We aim to determine the biological/ecological function(s) of sodorifen. The effects of sodorifen on bacteria (receiver and emitter) and fungi through genotype, proteome, and metabolome analysis will be performed. Sodorifen activity in growth and fitness of microbes can shed new light on the role of mVOCs.

**Keywords:** sodorifen, *Serratia plymuthica*, mVOCs

## References

Lemfack *et al.* 2018 *Nucleic acids research*

Piechulla, *et al.* 2017. *Plant, cell & environment*

Kai, *et al.* 2010. *Applied microbiology and biotechnology*

von Reuß, *et al.* 2010; 2016, *Angewandte Chemie International; American Chemical Society*

Schmidt, *et al.* 2017. *Scientific Reports*

Kai, *et al.* 2018. *FEMS microbiology letters*

## 204-EMV

### *In vivo* Mapping of the Mycosphere pH at the Microscale

\*B. J. Xiong<sup>1</sup>, C. Dusny<sup>2</sup>, K. Lindstaedt<sup>2</sup>, D. Schlosser<sup>1</sup>, L. Y. Wick<sup>1</sup>  
<sup>1</sup>Helmholtz-Center for Environmental Research UFZ, Environmental Microbiology, Leipzig, Germany

**Introduction:** The mycosphere is the microhabitat surrounding and affected by fungal hyphae. It forms an important ecological niche and is considered to be a hotspot of microbial activity. Little however is known, to what degree and at which scales hyphae shape this microbial habitat.

**Objectives:** We here present a novel, spatially and temporally resolved analysis of the influence of hyphae on the pH of mycosphere habitats at the microscale *in vivo*.

**Materials & methods:** The pH-sensitive fluorescent strain *Synechocystis* sp. PCC6803\_peripHlu was homogeneously embedded in an agar surface (pH: 6.9, size: 800 µm × 400 µm). The agar was then allowed to be overgrown by hyphae of the fungus *C. cinerea*. The pH change of individual bioreporter cells was quantified microscopically by optical ratiometric sensing and geostatistical approaches were applied for microscale mapping of the pH.

**Results:** Our data show that *C. cinerea* started to change the pH of a new habitat already at a distance of > 1.5 mm from the hyphal tip. Ongoing habitat colonization then changed the average pH of the agar from 6.9 to 5.8 within 18 h. Strikingly, spatial analysis of the agar, however, revealed the existence and permanence of a clearly distinct and patchy pH distribution and the presence of steep microscale pH gradients in the mycosphere between pH 4.4 and pH 5.5, respectively.

**Conclusion:** To our knowledge this is the first spatial explicit *in vivo* analysis of the mycosphere pH at the microscale. Such microscale pH mapping allows us to locate and quantitatively describe hyphal influences on the pH of their microhabitats. The presence of stable and steep pH gradients in the mycosphere is supposed to be a driver of fungal enzymatic activity and fungal bacterial interactions in the mycosphere.

## 205-EMV

Discovering a novel planctomycetal quorum sensing molecule

\*N. Kallscheuer<sup>1</sup>, O. Jeske<sup>2</sup>, B. Sandargo<sup>3,4</sup>, C. Boedeker<sup>2</sup>, S. Wiegand<sup>1</sup>, P. Bartling<sup>2</sup>, M. Jogler<sup>5</sup>, M. Rohde<sup>3</sup>, J. Petersen<sup>2</sup>, M. Medema<sup>6</sup>, F. Surup<sup>3,4</sup>, C. Jogler<sup>5</sup>

<sup>1</sup>Radboud University, Department of Microbiology, Nijmegen, Netherlands

<sup>2</sup>Leibniz Institute DSMZ, Braunschweig, Germany

<sup>3</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

<sup>4</sup>German Centre for Infection Research (DZIF), Braunschweig, Germany

<sup>5</sup>Friedrich-Schiller University Jena, Department of Microbial Interactions, Jena, Germany

<sup>6</sup>Wageningen University, Bioinformatics Group, Wageningen, Netherlands

Bacterial communication mostly relies on *N*-acyl homoserine lactones (AHLs), quorum sensing molecules that can trigger gene regulation in response to the density of a bacterial population. In contrast, unknown bacterial species of the human microbiome employ other *N*-acylated amino acids for cross-kingdom interaction with their host. During characterization of the bacterial isolate Mal15<sup>T</sup> belonging to the novel genus *Stieleria* in the phylum Planctomycetes, we were able to identify a novel class of *N*-acylated derivatives of the amino acid L-tyrosine. We named these compounds stieleriactines and showed similar quorum sensing effects as described for AHLs. During further elucidating the biosynthesis pathway for stieleriactines we found a potential

link to exopolysaccharide synthesis and analyzed the effects of stieleriactines on biofilm formation of Mal15<sup>T</sup> and faster-growing microorganisms competing with Mal15<sup>T</sup> for nutrient-rich biotic surfaces in marine environments. Based on the obtained results we postulate that stieleriactines can alter biofilm species composition in marine habitats, providing a growth advantage for the producer. Stieleriactines contribute to expand our knowledge on bacterial small molecule-mediated communication. While slow-growing Planctomycetes are known to dominate biofilms on marine biotic surfaces, stieleriactines might be used as biofilm scaping compounds, altering the bacterial species composition in such ecological niches. If stieleriactines serve for cross-kingdom communication, such as recently observed in the human gut, remains elusive.

## 206-NRZV

Update on diagnosis of Whipple's disease – from the gut over the heart valve into the urine?

J. Kikhney<sup>1</sup>, A. Wießner<sup>1</sup>, V. Moos<sup>2</sup>, M. Wolters<sup>3</sup>, H. Rohde<sup>3</sup>, \*A. Moter<sup>1</sup>

<sup>1</sup>Charité - Universitätsmedizin Berlin, Biofilmzentrum, Institut für Mikrobiologie und Infektionsimmunologie, Berlin, Germany

<sup>2</sup>Charité-Universitätsmedizin Berlin, Medizinische Klinik für Gastroenterologie, Infektiologie und Rheumatologie, Berlin, Germany

<sup>3</sup>Universitätsklinikum Eppendorf, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Hamburg, Germany

## Introduction

Whipple's disease (WD) is a rare infection with *Tropheryma whippelii* that is fatal if untreated. Diagnosis is challenging, and it is based mainly on invasive sampling. We previously reported on a kidney biopsy, where we observed morphologically intact bacteria within the glomerular capsular space and tubular lumen. This led to evaluation of polymerase chain reaction (PCR) testing of urine as a diagnostic test for WD.

## Objectives

Here we give insights into WD using fluorescence in situ hybridization (FISH) and report on the performance of urine testing for the diagnosis of WD.

## Materials & methods

In selected cases, we used FISH to localize *T. whippelii* directly within the patients' tissue. We prospectively investigated urine samples of 25 newly diagnosed and treated WD patients by PCR. As controls, we investigated samples from 110 healthy volunteers and patients with excluded WD or acute gastroenteritis.

## Results

Out of 25 urine samples from independent, therapy-naive WD patients, 19 were positive for *T. whippelii* PCR. In three patients, FISH visualized *T. whippelii* in urine. All control samples were negative, including those of 11 healthy carriers with *T. whippelii*-positive stool samples. In our study, the detection of *T. whippelii* in the urine of untreated patients correlated in all cases with WD.

## Conclusion

We show that *T. whippelii* is detectable by PCR in the urine of the majority of therapy-naive WD patients. With a low

prevalence but far-reaching consequences upon diagnosis, invasive sampling for WD remains mandatory, but must rely on a strong suspicion. Recent results from the Consiliary Laboratory strengthen the approach of urine testing as a novel, easy-to-obtain specimen for guiding the initial diagnosis of WD, in particular in patients with extra-intestinal WD. FISH sheds light on the invasiveness and biofilm potency of *T. whipplei* and confirmed a superinfection of a heart valve with *Cardiobacterium hominis*.

## 207-NRZV

### The jigsaw puzzle of genomics: Evaluation of sequencing and assembly strategies for *Francisella tularensis*, a re-emerging zoonotic pathogen

K. Neubert<sup>1,2</sup>, E. Zuchantke<sup>3</sup>, R. Leidenfrost<sup>4</sup>, R. Wünschiers<sup>4</sup>, H. Brendebach<sup>2</sup>, S. Al Dahouk<sup>2</sup>, H. Hotzel<sup>3</sup>, K. Reinert<sup>1</sup>, H. Neubauer<sup>3</sup>, H. Tomaso<sup>3</sup>, \*A. Busch<sup>5,3</sup>

<sup>1</sup>Freie Universität Berlin, Freie Universität Berlin, Department of Mathematics and Computer Science, Algorithmic Bioinformatics, Institut für Informatik, Berlin, Germany

<sup>2</sup>German Federal Institute for Risk Assessment, Berlin, Germany

<sup>3</sup>Friedrich-Loeffler-Institute, IBIZ, Jena, Germany

<sup>4</sup>University of Applied Sciences, Univer Department of Biotechnology and Chemistry, Mittweida, Germany

<sup>5</sup>University Hospital, KAI, Jena, Germany

## Introduction

*Francisella tularensis* is a highly virulent, Gram-negative bacterial pathogen and the causative agent of the zoonotic disease tularemia. Next-generation sequencing has arrived as a standard method generating short- or long-read raw sequences that are assembled to longer sequences for further analyses. Therefore, sequencing and assembly tools we evaluated these method before routine use.

## Objectives

Three objectives were assessed: (i) Analysis of the sequencing quality from five different sequencing approaches, (ii) optimal hybrid assembly techniques for long and short sequences were assessed to compare two approaches: long-read-first (with short read correction) (CANU/pilon, Flye) or short-read-first (with scaffolding on long reads) (Unicycler/ SPAdes). The third objective (iii) was to evaluate eight short-read assemblers.

## Materials & methods

We sequenced five diagnostically relevant *Francisella tularensis* strains isolated in Germany. using the short-read sequencers Ion Torrent's Ion S5, Illumina MiSeq, Illumina HiSeq and the long-read sequencers Pacific Biosciences RS (PacBio) and Oxford Nanopore Technologies Minion (ONT). Hybrid assembly was assessed to compare between the long-read-first (with short read correction) and short-read-first approach (with scaffolding on long reads) (CANU/pilon, Flye, Unicycler, SPAdes). The performance of short-read assembler (Abyss, IDBA, Masurca, Metaspades v3.9, Spades v3.11, Spades v3.9, Tadpole, Velvet) was compared.

## Results

Our quality assessment was based on sequencing data, sequencing error, coverage evenness, and GC-bias. All five sequencing platforms provided good sequencing results. Canu/pilon was most appropriate for PacBio-based hybrid assemblies with HiSeq data. The optimal short-read assembler was SPAdes 3.11.

## Conclusion

All sequencing platforms result in appropriate sequencing results to be used in epidemiological studies. For hybrid assembly, the method to use the long-read-first and correct then with short read had the best results for circularization and contiguity. Our results may help to better plan future sequencing projects of epidemiological studies.

## 208-NRZV

### Whole genome sequencing of *Neisseria meningitidis* W from Germany 2012 – 2018 during the European outbreak

\*M. Krone<sup>1</sup>, T. Läm<sup>1</sup>, U. Vogel<sup>1</sup>, H. Claus<sup>1</sup>

<sup>1</sup>Julius-Maximilians-Universität Würzburg, Institut für Hygiene und Mikrobiologie, Würzburg, Germany

## Question

While the number of invasive meningococcal disease (IMD) cases decreased in Germany and Europe over the last years, a rising incidence of IMD of serogroup W meningococci (MenW) caused by a unique hypervirulent clone of clonal complex (cc) 11 has been reported in several European countries (Krone, M., et al., *Increase of invasive meningococcal serogroup W disease in Europe, 2013 to 2017*. Euro Surveill, 2019. **24**(14)). The UK Strain 2013 was responsible for the change in European epidemiology (Lucidarme, J., et al., *An international invasive meningococcal disease outbreak due to a novel and rapidly expanding serogroup W strain, Scotland and Sweden, July to August 2015*. Euro Surveill, 2016. **21**(45)). The aim of the study is to describe MenW epidemiology in Germany from 2002 – 2018. To analyse the whole genome sequencing (WGS) the population structure of German MenW strains.

## Methods

Laboratory surveillance data collected by the National Reference Laboratory for Meningococci and Haemophilus influenzae (NRZMHI) and statutory data of the Robert Koch Institute were analysed. WGS was done at the core unit systems biology of the University of Würzburg on an Illumina NextSeq 500 sequencer using the Nextera XT library preparation. SeqSphere+ software (Ridom) was used for de novo assembly (Velvet) and allele calling for cgMLST assignment. BIGSdb and SeqSphere were used for phylogenetic analyses.

## Results

While the share of MenW on all IMD cases in Germany had ranged from 1 to 4 % before 2016, an increase in MenW IMD incidence and proportion on all IMD was observed since 2016 reaching 13 % of all IMD and 0.04 cases per 100,000 population in 2018. While MenW cases between 2012 and 2015 were mainly caused by cc22 and cc23, an expansion of cc11 could be observed since 2016 of which the majority of the cases in 2017 (10/14) and 2018 (14/19) were caused by the "UK 2013" strain.

## Conclusions

The increase of MenW cases mainly caused by the "UK 2013" strain in Germany follows developments in other European countries. However, in contrast to the UK and the Netherlands, this increase is very moderate and an epidemiologically relevant expansion cannot be confirmed yet.

---

## 209-NRZV

### The rise of *Enterococcus faecium* ST117 - a 20 years longitudinal analysis of a successful hospital-adapted lineage

\*J. Bender<sup>1</sup>, C. Fleige<sup>2</sup>, B. Neumann<sup>2</sup>, R. Weber<sup>2</sup>, I. Klare<sup>2</sup>, G. Werner<sup>2</sup>

<sup>1</sup>Robert Koch Institute, Department of Infectious Diseases, Berlin, Germany

<sup>2</sup>Robert Koch Institute, Division 13 Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany

**Background:** Enterococci are considered the second to third most common cause of nosocomial infections. Especially vancomycin-resistant *Enterococcus* spp. (VRE) pose a serious threat to health care institutions as limited treatment options exist for those pathogens. Vancomycin resistance is most prominent in *E. faecium* of which hospital-adapted lineages have emerged. Multi-locus sequence typing (MLST) has identified lineage ST117 amongst the *E. faecium* population that is highly prevalent in German hospitals nowadays. In order to disclose population dynamics and distribution of the resistance genotype over time, we analysed a comprehensive strain collection of *E. faecium* ST117 received by the NRC from 1998 until 2018.

**Methods:** In total, 415 *E. faecium* ST117 of various clinical materials and including outbreak isolates were subjected to whole-genome sequencing (WGS). Phylogenetic relatedness was assessed by core genome MLST (cgMLST) using the SeqSphere® software. The pan-genome was analysed using Roary and Scoary.

**Results:** WGS and cgMLST analyses allowed sub-differentiation of the ST117 population into 81 distinct complex types (CT). A plethora of novel CTs were identified; however, a few CTs such as CT71 dominate the *E. faecium* ST117 population and were causative for outbreaks. Most interestingly, specific CT dynamics were observed over the study period of 20 years, indicating the rise and fall of distinct sub-lineages in German hospitals. Further bioinformatics investigations demonstrate a clear association of either *vanA* or *vanB* with selected CT-lineages and the emergence of *vanB* in *E. faecium* ST117 in recent years. Gene distribution cluster analysis revealed a partially lineage-specific gene repertoire.

**Conclusions:** Our analyses suggest that the emergence, dissemination and the decline of hospital-adapted *E. faecium* ST117 clinical isolates is highly dynamic but simultaneously dominated by selected clonal lineages. Detailed molecular investigations are currently in progress to obtain insights into the genetic basis that might facilitate certain strain types to emerge as successful pathogens in clinical settings.

---

## 210-NRZV

Molecular surveillance of listeriosis in Germany: Identification of large listeriosis outbreaks by whole genome sequencing

\*S. Halbedel<sup>1</sup>, H. Wilking<sup>2</sup>, S. Kleta<sup>3</sup>, A. Holzer<sup>2</sup>, R. Lachmann<sup>2</sup>, M. Fischer<sup>1</sup>, A. Pietzka<sup>4</sup>, S. Lüth<sup>3,5</sup>, S. Huhulescu<sup>4</sup>, S. Al Dahouk<sup>3,6</sup>, K. Stark<sup>2</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>Robert Koch-Institute, FG11 - Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany

<sup>2</sup>Robert Koch Institute, FG35 Division of Gastrointestinal Infections, Berlin, Germany

<sup>3</sup>German Federal Institute for Risk Assessment, Berlin, Germany

<sup>4</sup>Austrian Agency for Health and Food Safety, Wien, Austria

<sup>5</sup>Freie Universität Berlin, Berlin, Germany

<sup>6</sup>RWTH Aachen, Aachen, Germany

**Introduction:** *Listeria monocytogenes* causes foodborne infections with high mortality. The majority of cases are either sporadic or occur in small disease clusters. However, large outbreaks may occasionally arise, leading to a significant burden of disease.

**Objectives:** *L. monocytogenes* isolates from approximately two-thirds of all mandatorily notified German listeriosis cases are sent to the bi-national Consiliary Laboratory for *Listeria* at the Robert Koch Institute and the Austrian Agency for Health, which run a subtyping program for identification of listeriosis outbreak clusters.

**Materials & Methods:** Whole genome sequencing (WGS) and core genome multi locus sequence typing (cgMLST) were used for subtyping of more than 1,700 human *L. monocytogenes* isolates collected in Germany between 2015 and 2019. Presumable food vehicles for listeriosis infections were identified through matching of cgMLST subtyping results from human isolates and food isolates collected by the National Reference Laboratory at the German Federal Institute for Risk Assessment. For selected cgMLST clusters, patients were interviewed on their food consumption habits to add epidemiological evidence.

**Results:** Two thirds of all human isolates grouped into more than 160 different cgMLST clusters. Among them were several large clusters including one with 112 cases, which has been among the largest European listeriosis outbreaks for more than 25 years. For some clusters, case-control studies and WGS-typing of isolates from suspected food sources provided the key for identification of the source of infection.

**Conclusions:** Our work illustrates the importance and suitability of a comprehensive WGS-based national surveillance system comprising human and food isolates in order to stop and to prevent listeriosis outbreaks.

---

## 211-NRZV

High heterogeneity of plasmid-mediated quinolone resistance in *Escherichia coli* isolates recovered from livestock and food in Germany

\*K. Juraschek<sup>1</sup>, M. Grobbel<sup>1</sup>, A. Käsbohrer<sup>1</sup>, B. A. Tenhagen<sup>1</sup>, J. A. Hammer<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany

**Introduction:** Resistance to quinolones can be chromosomally encoded or plasmid-mediated (PMQR). One PMQR mechanism is mediated by Qnr proteins. The horizontal gene transfer of this plasmid-mediated quinolone resistance increases the threat of fallible treatment with quinolones.

**Objectives:** To better understand the distribution of PMQR, in particular *qnr* genes, *Escherichia* (*E.*) *coli* isolates recovered in 2017 from livestock and food were phenotypically and genotypically characterized.

**Materials & Methods:** 3,409 *E. coli* isolates from the German National Reference Laboratory for Antimicrobial Resistance were investigated. The isolates were received in the German national monitoring program for antimicrobial resistance. Antimicrobial resistance was determined by broth microdilution according to CLSI guidelines. MIC values for ciprofloxacin and nalidixic acid were evaluated using EUCAST epidemiological cut-off values (MIC<sub>NAL</sub> ≥16 mg/L, MIC<sub>CIP</sub> ≥0.06 mg/L). *E. coli* resistant to quinolones were

subjected to *qnr*-PCR, XbaI-PFGE, S1-PFGE, WGS and bioinformatic analysis. Six different *qnr*-PCRs were conducted to identify the respective *qnr*-variants.

**Results:** Overall, 504 isolates were classified as quinolone-resistant. Of those, 107 were found to harbor a *qnr* gene. The most abundant *qnr*-variant was *qnrS*. PFGE profiling for the 107 *qnr* positive isolates demonstrated a high heterogeneity, indicating that they are not associated to a predominant *E. coli* clone spreading via vertical transmission. S1-PFGE plasmid profiling showed a variety of extrachromosomal elements of various sizes. 43 Isolates, selected according their XbaI- and S1-PFGE pattern were further screened for their genetic setting through short read whole genome sequencing (WGS). Sequencing confirmed the high genetic diversity of the quinolone-resistant *E. coli* strains.

**Conclusion:** Quinolone-resistance could not be attributed to a specific lineage of *E. coli*. Further analysis is needed for a better understanding of the plasmid diversity within *qnr*-harboring *E. coli* and the prerequisites of their spread.

---

## 212-NRZV

Causative agents of invasive fungal infections in Germany and their antifungal susceptibilities determined by the National Reference Centre for Invasive Fungal Infections (NRZMyk)

\*G. Walther<sup>1</sup>, O. Kurzai<sup>1,2</sup>

<sup>1</sup>HKI, NRZMyk, Jena, Germany

<sup>2</sup>University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany

The National Reference Centre for invasive fungal infections (NRZMyk) receives fungal isolates and native clinical materials from hospitals and registered doctors across Germany. All isolates are identified molecularly by the use of makers that are discriminative for the respective taxa. Antifungal susceptibility testing is performed *in vitro*, by microdilution testing according to the EUCAST protocol for most isolates. Additionally, molecular testing is performed for echinocandin resistance of *Candida* spp. and azole resistance of *Aspergillus fumigatus*. In *Aspergillus*, molecular identification revealed that clinical isolates morphologically identified as *Aspergillus niger* or *Aspergillus nidulans* in most cases represent different species with deviant antifungal susceptibility profiles. Species morphologically similar to *Aspergillus fumigatus* but with intrinsic antifungal resistances such as *A. lentulus* or *A. udagawae* are rarely observed by the NRZMyk. In *Aspergillus fumigatus*, azole resistance is seen in two thirds of the strains and is caused by mutations of the 14- $\alpha$ -sterol demethylase gene (CYP51A). The most frequent mutation is TR34/L98H. In the last five years, the isolates of *A. fumigatus* received by the NRZMyk did not show a significant increase in the azole resistance. Importantly, the NRZMyk did not detect any increases in rare *Candida* spp. such as *Candida auris*. Echinocandine resistance in *C. albicans* and *C. glabrata* is often caused by mutations of the target genes, the 1,3- $\beta$ -D-glucan synthase genes and can be acquired within short periods of treatment. Molecular species identification in combination with antifungal susceptibility testing contributes to an improved management of fungal infections.

---

## 213-NRZV

**Report of the German Consulting Laboratory on Tick-Borne Encephalitis**

\*G. Dobler<sup>1</sup>

<sup>1</sup>Bundeswehr Institute of Microbiology, Virology and Rickettsiology, München, Germany

Tick-borne encephalitis (TBE) is the most important tick-transmitted viral infection in Europe and Asia. In Germany very high numbers of registered human cases were reported in Germany, with 486 cases in 2017 and 585 human cases in 2018. In 2018, the highest number of ticks within the last 10 years was sampled in a representative TBE natural focus areas in Germany. The geographical distribution of TBE cases in Germany showed that the high numbers of human TBE cases are due to increases in the known endemic areas. Newly emerging TBE endemic areas contribute only to a small proportion to the increase of human cases seen during the last two years. Therefore, an association between tick numbers and human TBE cases was assumed. To test this hypothesis ticks were sampled in 2019 monthly in a control TBE natural focus area and human cases reported to the Robert Koch-Institute were associated. A total of 1,638 ticks (104 males, 75 females, 1,459 nymphs) were sampled. In comparison with the year 2018 (1,875 ticks, 131 males, 104 females, 1,640 nymphs) the number of ticks was only about 10% higher than in 2019. However, the number of human TBE cases has decreased about 30% from 2018 to 2019 (estimated 400 human cases). In 2018 the minimal infection rate (MIR) in the tick population was 0.16% (3/1,875 ticks). In 2019 the MIR was calculated as 0.24% (4/1,638). The data show that the absolute number of ticks and the MIR in ticks do not correlate with the number of human TBE cases. These results imply that mainly anthropogenic activities determine the number of human cases. Most cases still occur in well-known TBE risk districts while human TBE cases from emergent risk areas are responsible for a small proportion of cases.

---

## 214-MSHV

**Metamorphosis of the marine Hydrozoan *Hydractinia echinata* is mediated by bacterial membrane components**

\*M. Rischer<sup>1</sup>, H. Guo<sup>1</sup>, C. Beemelmans<sup>1</sup>, M. Westermann<sup>2</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute (HKI), Chemical Biology of Microbe-Host Interactions, Jena, Germany

<sup>2</sup>University Hospital Jena, Electron Microscopic Center, Jena, Germany

## Question

Bacterial signalling substances induce colonisation and metamorphosis in larvae of marine invertebrates such as sponges, cnidarians, mollusca and annelids [1]. We have chosen the marine polyp *Hydractinia echinata* as a model system to study the initiation of metamorphosis and the chemical communication between the eukaryotic host and the associated microbiome [2]. The life cycle of the hydroid polyp *H. echinata* includes the bacterial induced transition of the mobile larvae into the sessile reproduction phase (polyp). The larva develops into a primary polyp only in response to a chemical cue/specific molecule provided by associated environmental bacteria.

## Methods

We used a culture-dependent and independent approach to identify associated microbes. The isolated strains were investigated for their potential to induce metamorphosis of *H. echinata* larvae using a stable monospecies induction assay. Strains that reliably induced metamorphosis were prioritized and different purification methods were applied to isolate the involved substances by bioassay guided fractionation.



## Results

Several results indicated that more than one signaling substance is involved in the morphogenesis process. Further chemical analysis of active fractions caused the identification of chemically distinct types of morphogenic metabolites including certain bacterial membrane components, such as different phospholipids. Fluorescence measurement showed that synthetic phospholipid derivatives are incorporated into the cell membrane of the larvae. Likewise, a polymeric sugar, containing rhamnose-mannose units, induced full metamorphosis [3].

## Conclusions

Overall, our results suggest that the metamorphic transition of the motile larva to the sessile polyp needs to be considered as an orchestrated, multi-factorial or even synergistic event, which requires most likely different bacterial-produced signaling cues.

## References

- [1] Cantley AM, Clardy J (2015) *Nat. Prod. Rep.*, 32, 888–892.
- [2] Müller WA, Leitz T (2002) *Can. J. Zoo.*, 80, 1755–1771.
- [3] Guo H, Rischer M, Sperfeld M, Weigel C, Menzel KD, Clardy J, Beemelmans C (2017) *Bioorg. Med. Chem.*, 25, 6088–6097.

### 215-MSHV

The RWV bioreactor: A 3D cell culture model to characterize the molecular mechanisms of bacterial (probiotic *E. coli* Nissle 1917) and host interaction

\*J. Gonzalez<sup>1</sup>, R. von Buenau<sup>2</sup>, P. Dersch<sup>1</sup>, U. Dobrindt<sup>3</sup>, C. Cichon<sup>1</sup>

<sup>1</sup>Center for Molecular Biology of Inflammation, Institute of Infectiology, Muenster, Germany

<sup>2</sup>Pharma-Zentrale GmbH, Herdecke, Germany

<sup>3</sup>Institute of Hygiene, Muenster, Germany

**Background:** The increase of antibiotic resistance making infectious diseases a major cause for death worldwide, provokes an urgent need for the development of novel therapeutic strategies (e.g. preventive probiotics). For decades, two-dimensional cell culture provided the best way to study bacterial-host interactions *in vitro*. Since the 1990s, there has been a shift from the "flat biology" to advanced 3D cell culture methods of intestinal epithelia, closely mimicking the microenvironment for host-microbe crosstalk, offering "*in vitro*" tools for the analysis of cellular response (e.g. barrier function, protective mucin production) to infection.

**Objectives:** Advanced cell culture models are necessary to fast-track *in vitro* based research to complex *in vivo* models for infectious diseases.

**Material & Methods:** Employing the colonic cell lines HT29, HT29-MTX and LS174T we compared cellular responses to co-incubation with the probiotic *E. coli* Nissle 1917 and flagella variants. Corresponding gene expression profiles of cell lines were compiled (RT2 Profiler Arrays (Qiagen)). Epithelial cells on Transwell® Inserts do polarize but do not mimic a complex mucosal surface. This made us to use the rotating wall vessel (RWV) bioreactor, engineered to study cellular and molecular responses of host and microbes. It offers dynamic culture conditions for epithelial cells like low

fluid shear stress influencing gene regulation and cellular differentiation of cells growing on Cytodex-3 beads.

**Results:** Our studies of bacterial-host interaction revealed obvious differences between varying cell culture models concerning: a) bacterial induced fine tuning of host responses by miRNA regulation, e.g. TNF, MyD88 (immune response) by miR-181a or miR-200c, JamA (barrier function) by miR-320a, b) the impact of a protective apical mucin layer and c) rotating infection conditions (bacterial adhesion).

**Conclusions:** These differences shed light on mechanisms governing host-microbe (probiotic) interactions, delivering more physiologically relevant results about intestinal disease development and pave the way for the translation "from bench to bedside" (application of probiotics).

### 216-MSHV

Predatory bacteria enhance microbiome diversity and host health

\*J. Johnke<sup>1</sup>, H. Schulenburg<sup>1</sup>

<sup>1</sup>CAU Kiel, Evolutionary Ecology and Genetics, Kiel, Germany

## Question:

Microbial diversity positively correlates with ecosystem stability. This relationship also applies to host-associated microbiomes. Therefore, factors contributing to microbial diversity are of great interest, especially as certain diseases are linked to microbiome dysbiosis. A so far understudied group of candidates for microbiome restoration are predatory bacteria belonging to the *Bdellovibrio*- and like organisms (BALOs). These bacteria can be considered as probiotic (they reduce dominant species, thereby promoting less abundant species) as well as antibiotic (they kill other bacteria). A previous meta-analysis of microbiomes from distinct hosts revealed that BALO presence associates with a higher microbiome alpha-diversity. BALOs might therefore prevent microbiome dysbiosis by actively preying on dominant species, thus increasing host health.

## Methods:

In order to elucidate if BALOs are drivers of microbiome diversity we performed controlled laboratory experiments in culture as well as *in vivo* using the nematode *Caenorhabditis elegans* as model host. We assembled microbiome communities of different complexity and tracked the community dynamics in the presence or absence of a newly isolated BALO over two weeks via 16S rRNA gene sequencing. Host brood size and population growth were measured as proxies for host health and fitness.

## Results:

We found a general decrease in community richness over time, but this decrease was significantly delayed in the presence of the predatory bacterium. Additionally, specific microbiome members became significantly more abundant in cultures containing the BALO and showed different co-occurrence pattern than in BALO-free cultures. Finally, we found a positive association between BALO presence and *C. elegans* health and fitness.

## Conclusions:

Our results demonstrate that BALOs have a positive effect on microbial diversity and potentially host health and

therefore represent promising candidates for the restoration of disturbed microbiomes.

---

## 217-MSHV

Dual-Seq of *Caedibacter taeniospiralis* genome and transcriptome, obligate endosymbiont of *Paramecium*  
M. Pirritano<sup>1</sup>, N. Zaburannyi<sup>2</sup>, K. Grosser<sup>3</sup>, G. Gasparoni<sup>4</sup>, R. Müller<sup>2</sup>, M. Simon<sup>1</sup>, \*M. Schrällhammer<sup>3</sup>

<sup>1</sup>Wuppertal University, Wuppertal, Germany

<sup>2</sup>Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Department of Microbial Natural Products, Saarbrücken, Germany

<sup>3</sup>Albert-Ludwigs University of Freiburg, Microbiology, Freiburg, Germany

<sup>4</sup>Saarland University, Genetics, Saarbrücken, Germany

Interest in host-symbiont interactions is continuously increasing, not only due to the recognized importance of microbiomes. Started with the detection and description of novel symbionts, attention moves to the molecular consequences and innovations of symbioses. Molecular analysis requires genomic data that is difficult to obtain from obligate intracellular and uncultivated bacteria. How do we obtain the genome and transcriptome of the obligate endosymbiont *Caedibacter taeniospiralis*, a member of the little studied group *Fastidiosibacteriaceae*? This symbiont confers its unicellular host *Paramecium tetraurelia* with the ability to kill other cells, and with immunity against this effect. Based on the genomic data, which new insights are gained into the establishment and consequences of this complex modified killer phenotype?

Paramecia were cultivated with hypersensitive *E. coli* allowing to remove food bacteria from the culture before DNA and RNA extraction without the risk of eliminating the symbiont. We obtained the *C. taeniospiralis* genome and transcriptome by dual-Seq of DNA and RNA from infected paramecia.

Comparison of codon usage and expression level indicates that genes necessary for a specific trait of this symbiosis, i.e. the delivery of an unknown toxin, result from horizontal gene transfer hinting to the relevance of DNA transfer for acquiring new characters. Prediction of secreted proteins of *Caedibacter* as major agents of contact with the host implies, next to several toxin candidates, a rather un-characterized secretome that appears to be highly adapted to this symbiosis.

Our data provides new insight into the molecular establishment and evolution of this obligate symbiosis and for the pathway characterization of toxicity and immunity.

---

## 218-MSHV

**Priority effects in *Pantoea agglomerans* colonization of the wheat root**

\*R. Soluch<sup>1</sup>, N. Hülter<sup>1</sup>, T. Dagan<sup>1</sup>

<sup>1</sup>Christian-Albrechts-Universität zu Kiel, Institut für Allgemeine Mikrobiologie, Kiel, Germany

Eukaryotes, including plants, are hosts to microbes. These microbes can be recruited from the environment as well as transferred between the generations horizontally or vertically. We hypothesize, that in wheat, due to priority effects, vertically transferred microbiota has priority advantage over bacteria recruited from the environment.

Isolation efforts on *Triticum aestivum* seeds and seedlings have yielded few cultivable bacterial strains present both on

and in the seed and seedling. *Pantoea agglomerans* was the species found in most of the samples, independent of the geographical origin (Germany, Turkey).

Here we show that GFP-tagged strain of *Pantoea agglomerans*, originally isolated from wheat, maintains its ability to colonize germ-free wheat roots and to proliferate within the root. Independently of the inoculum size used, log-phase-like population growth within the root was observed. Afterward, while population growth was still ongoing, any further increase in population size was tied to the growth of the root habitat. Once population count was standardized based on root fresh weight, the plateau was observed within 8 days post-inoculation, showing that the carrying capacity of the host is limited.

*In planta* competitions between GFP and mCherry tagged *Pantoea* strains were performed to check for numerical priority effects and adaptive priority effects. Competing strains were introduced with a time lag between them or one of the strains was pre-cultivated in a plant, while the second was cultivated in liquid media.

Our results show a strong impact of the priority effect. The numerical advantage of primary colonizer led to its high relative abundance. No adaptive advantage was observed. Compared to our single colonizer experiments, total carrying capacity of the host was unchanged.

This data provides the fundament for future experiments on mechanistic aspects of wheat colonization by *Pantoea agglomerans*, which can be performed by analysis of the bacterial genome and preparation of knock-outs. The established gnotobiotic system enables further competition and exclusion experiments.

---

## 219-MSMV

**Deciphering the lifestyle of Pseudomonads and related species using a novel high resolution <sup>13</sup>C metabolic flux approach**

\*M. Kohlstedt<sup>1</sup>, C. Wittmann<sup>1</sup>

<sup>1</sup>Saarland University, Institute of Systems Biotechnology, Saarbrücken, Germany

## Introduction

Pseudomonads exhibit a complex metabolic architecture and are known to assimilate glucose via three convergent peripheral pathways [1]. After uptake into periplasm, glucose can be either phosphorylated directly into glucose 6-phosphate or it can be oxidized into gluconate, which is then taken up in the cytoplasm and phosphorylated. In addition, gluconate can be further converted into 2-ketogluconate, which is transported into the cytoplasm, subsequently phosphorylated and finally reduced via the 2-ketogluconate loop. All three catabolic steps converge at the level of 6-phosphogluconate, which is next cleaved into glyceraldehyde 3-phosphate and pyruvate via the Entner-Doudoroff pathway. These trioses can then be recycled back into hexose phosphates or further channeled into lower carbon metabolism.

## Objectives

We present a simple, robust and solely GC-MS-based approach to resolve the complex metabolic architecture of Pseudomonads as demonstrated for *P. putida* KT2440 and *P. aeruginosa* PAO1.

## Materials & Methods

For high resolution  $^{13}\text{C}$  metabolic flux analysis, we combined parallel labeling experiments on different  $^{13}\text{C}$  tracers with GC-MS measurements of labeling data obtained from proteinogenic amino acids, cell glycogen and peptidoglycan [2].

## Results

Additional information for glucose 6-phosphate and fructose 6-phosphate from cell glycogen and glucosamine resulted in an extensive data set with sufficient power to resolve all relevant fluxome features in the investigated bacteria. Excellent fits between experimental and simulated data were obtained. Both strains reveal a high metabolic flexibility towards different carbon sources and stressors.

## Conclusion

Our novel approach allows studying the metabolism of Pseudomonads to great detail (including more than 250 members of biotechnological [3] and clinical relevance [4]). Also other microbes, exhibiting a similar pathway architecture, now become accessible on the flux level.

## References

- [1] del Castillo *et al.* (2007) J. Bacteriol. 189, 5142-52
- [2] Kohlstedt & Wittmann (2019) Metab. Eng. 54:35-53.
- [3] Kohlstedt *et al.* (2018) Metab. Eng. 47:279-293.
- [4] Berger *et al.* (2014) PLOS One 9:e88368.

## 220-MSMV

### Genome-scale microbial community modeling with $\mu\text{bialSim}$

\*F. Centler<sup>1,2</sup>, D. Popp<sup>1,2</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>UFZ, Department of Environmental Microbiology, Leipzig, Germany

Microbial communities are ubiquitous in nature and impact human well-being in many ways. Due to their complexity with respect to their spatio-temporal dynamics and the intricate interaction networks driving these dynamics, they are notoriously difficult to understand. Modeling has been applied as a valid tool to untangle ecological interactions in these systems but usually not resolving intracellular metabolic fluxes. These are explicitly considered in constraint-based methods which are a standard tool for monocultures, typically relying on genome-scale metabolic networks of sequenced microbial species.

To characterize microbiome dynamics both on the species abundance and down to the enzyme-level, we here introduce  $\mu\text{bialSim}$  (pronounced "microbialSim"), a novel numerical simulator that implements the dynamic Flux-Balance-Analysis approach and expands it to communities. By employing a novel numerical integration scheme, our simulator can consider communities at their natural diversity, going beyond current simulator capabilities which are restricted to few species or low abundance communities.

As an example, we apply  $\mu\text{bialSim}$  to the entirety of a model collection of 773 species of the human gut microbiome. We demonstrate how the predicted pattern of compound exchange and its dynamics can be analyzed as the community feeds on a western-diet substrate pulse.

While quantitative predictions have to be interpreted in the light of the simulator's current limitations – being restricted to metabolic interactions only – we envision  $\mu\text{bialSim}$  as a starting point for an extensive *in silico* characterization of community dynamics at an unprecedented level of detail. It can be used as a tool for experimental design and the design of communities for novel biotechnological applications. Combined with OMICS data on enzyme activity it will be an invaluable tool to decipher activity in experimental microbiomes and helping in elucidating general principles in microbial ecology.

## 221-MSMV

### Observation of fluorescent FAST-tagged protein production during growth of the strict anaerobic acetogen *Eubacterium limosum*

\*M. Flaiz<sup>1</sup>, J. Gaibler<sup>1</sup>, F. R. Bengelsdorf<sup>1</sup>, P. Dürre<sup>1</sup>

<sup>1</sup>Ulm University, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany

**Introduction:** Fluorescent proteins are indispensable for several applications in molecular biology. Commonly, the green fluorescent protein and its derivatives are used, but their usage is limited to aerobic bacteria, since chromophore maturation requires oxygen. The fluorescence-activating and absorption-shifting tag (FAST) is a fluorescence-activating protein and only shows fluorescence when bound to a fluorogenic ligand. Its fluorescence is independent of oxygen and therefore perfectly suited as a molecular biological tool in anaerobic bacteria such as *Eubacterium limosum*. *E. limosum* is an acetogen, which is genetically accessible and a promising biocatalyst for the sustainable production of chemicals from C1 carbon sources. The establishment of molecular tools using the FAST protein is of great interest.

**Objectives:** In this study, the functionality of FAST as a reporter was evaluated in *E. limosum* by expression using different promoters and by fusion to the protein 4-hydroxybutyryl-CoA dehydratase (AbfD).

**Materials and Methods:** FAST was cloned under the control of the different constitutive and inducible promoters  $P_{thi}^{sup}$ ,  $P_{ptaack}$ ,  $P_{tet}$ , and  $P_{bgaL}$ . In addition, the plasmid pMTL83251\_  $P_{bgaL}$ -C-FAST-abfD was constructed, which encodes the FAST-tagged *abfD* gene. *E. limosum* was electroporated using the resulting plasmids, and recombinant strains were analysed by measuring FAST dependent fluorescence during growth.

**Results:** *E. limosum* [pMTL83251\_  $P_{bgaL}$ -FAST] showed intense fluorescence when gene expression was induced. In the absence of lactose, however, no fluorescence was measured. The inducible promoter system *bgaR*- $P_{bgaL}$  seems to be tightly regulated in *E. limosum* and causes strong gene expression when induced, making it perfectly suitable for the construction of production plasmids. During growth of *E. limosum* [pMTL83251\_  $P_{bgaL}$ -C-FAST-abfD], fluorescence increased after induction of gene expression. This indicates that the fusion protein and therefore AbfD was produced.

**Conclusion:** FAST is a promising molecular biological tool in anaerobic bacteria. It is suitable as reporter for promoter studies and as a fluorescent tag for fusion protein construction.

---

## 222-MSMV

### Modelling phototrophic growth: from single cells to microbial communities

\*R. Steuer<sup>1</sup>

<sup>1</sup>Humboldt-Universität zu Berlin, Institut für Theoretische Biologie (ITB), Berlin, Germany

**Introduction:** Microbial life is ubiquitous in all environments on Earth, and understanding the life and growth of microbes has outstanding relevance for biotechnology, biogeochemistry, and human health. The construction of computational models is highly useful tool to understand the organization and functioning of microbial metabolism.

**Objectives:** The purpose of this contribution is to outline recent advances in modelling microbial growth from the perspective of cellular resource allocation, with a focus on cyanobacterial phototrophic growth.

**Results:** Over the past years, we developed a mathematical framework that describes cyanobacterial growth based on coarse-grained models of cellular proteome allocation. Of particular interest are the resulting microbial 'growth laws', i.e., the correlations between the growth rate and the proteome distribution observed during balanced growth. The models predict a characteristic kink for the growth laws of the light harvesting components induced by photoinhibition, the predicted growth laws are in good agreement with quantitative mass spectrometry-based proteomics data of the cyanobacterium *Synechocystis* sp. PCC 6803.

**Conclusion:** Coarse-grained microbial growth models are physiologically meaningful while remaining computationally tractable. Such models allows us to represent the physiological acclimation of cyanobacteria to different environments, co-limitation of growth by several nutrients, as well as emergent metabolic switches between alternative nutrient sources. Our next step is therefore to understand the interactions and dynamics within microbial communities and to construct predictive models of microbial growth for ecosystems simulations.

---

## 223-MSMV

### Lights on and action – optogenetic control of biosynthetic pathways

\*F. Hilgers<sup>1</sup>, N. Bitzenhofer<sup>1</sup>, D. Binder<sup>1</sup>, A. Burmeister<sup>2,3</sup>, A. Grünberger<sup>2,3</sup>, A. Loeschcke<sup>4</sup>, F. Hogenkamp<sup>5</sup>, J. Pietruszka<sup>5,6</sup>, K. E. Jaeger<sup>6,7</sup>, T. Drepper<sup>1</sup>

<sup>1</sup>Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Forschungszentrum Jülich, Bacterial Photobiotechnology, Jülich, Germany

<sup>2</sup>Institute of Bio- and Geosciences IBG-1, Forschungszentrum Jülich, Multiscale Bioengineering, Jülich, Germany

<sup>3</sup>University Bielefeld, Multiscale Bioengineering, Bielefeld, Germany

<sup>4</sup>Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Forschungszentrum Jülich, Natural product biosynthesis, Jülich, Germany

<sup>5</sup>Institute of Bioorganic Chemistry, Heinrich Heine University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

<sup>6</sup>Institute of Bio- and Geosciences IBG-1, Forschungszentrum Jülich, Jülich, Germany

<sup>7</sup>Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

Optimized microbial production processes often require precise control over targeted metabolic pathways and underlying regulatory networks. In recent years, optogenetic tools such as light-responsive switches have been implemented which enable a non-invasive control over cellular functions with high spatiotemporal resolution. Here,

we report on the development and evaluation of different optogenetic *on* and *off* switches that allow for light-mediated control of gene expression or protein activity and demonstrate the applicability of these switches for programming bacterial production processes with light.

**Optogenetic *on* switches:** Broadly applicable light-responsive expression systems were developed by employing photocaged inducer molecules like caged IPTG and arabinose<sup>1,2</sup>. Light-induced cleavage of the photosensitive protection group results in an intracellular release of inducer molecules and an immediate target gene expression. The biotechnological applicability of these phototriggers could be demonstrated by optimizing the synthesis of secondary metabolites such as violacein in *E. coli*<sup>2</sup>. Furthermore, the toolbox of photocaged compounds provides inducer molecules with differing chemical properties like e.g. water solubility and enables the expression in different host strains.

**Optogenetic *off* switches:** Genetically encoded photosensitizers (PS) are proteins that produce reactive oxygen species upon illumination<sup>3,4</sup>. Due to this feature, PS constitute suitable optogenetic tools for chromophore-assisted light inactivation (CALI) of target enzymes in living bacteria. By using the bifurcated biosynthetic pathway of the antibiotic tripyrrole prodigiosin, we could demonstrate gradual PS-mediated inhibition of the final condensing enzyme PigC.

Because of their unique properties, these newly established optogenetic switches can be applied in the near future as versatile plug-and-play tools suitable for regulating and optimizing complex production processes by light in a broad range of different bacteria.

<sup>1</sup>Binder *et al. Integr. Biol.*, 6(6):755–765.

<sup>2</sup>Binder *et al. ChemBioChem*, 17(4):296–299.

<sup>3</sup>Endres *et al. Sci. Rep.*, 8(1):15021.

<sup>4</sup>Hilgers *et al. Int. J. Mol. Sci.*, 20(18):4608.

---

## 224-MSMV

### Marburg Collection: A highly flexible cloning toolbox for the emerging SynBio chassis *Vibrio natriegens*

\*D. Stukenberg<sup>1</sup>, T. Hensel<sup>2</sup>, J. Hoff<sup>1</sup>, B. Daniel<sup>1,3</sup>, R. Inckemann<sup>4</sup>, G. Fritz<sup>5</sup>

<sup>1</sup>LOEWE Center for Synthetic Microbiology, Marburg, Germany

<sup>2</sup>Philipps-University, Chemistry, Marburg, Germany

<sup>3</sup>ETH Zuerich, Biology, Zuerich, Germany

<sup>4</sup>Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany

<sup>5</sup>University of Western Australia, School of Molecular Sciences, Perth, Australia

*Vibrio natriegens* is known as the world's fastest growing organism with a doubling time of less than 10 minutes. This incredible growth speed proposes *V. natriegens* as a chassis for synthetic and molecular biology and for replacing *E. coli* in many applications. So far, many foundational genetic tools, e.g. inducible promoters, plasmid replication origins or reporter genes, are not yet established or not characterized in *V. natriegens* and therefore the widespread use of this promising chassis is prevented. To overcome this limitation, we created the Marburg Collection, a highly flexible Golden Gate-based cloning toolbox optimized for the emerging chassis organism *V. natriegens*.

The Marburg Collection overcomes the paradigm of plasmid construction – integrating inserts into a backbone – by enabling the *de novo* assembly of plasmids from basic genetic parts. This allows users to select the plasmid replication origin and resistance part independently, which is highly advantageous when working with limited knowledge about the behavior of those parts in the target organism. Additional design highlights of the Marburg Collection are novel connector parts which facilitate modular circuit assembly and, optionally, the inversion of individual transcription units to reduce crosstalk in multigene constructs.

To quantitatively characterize the genetic parts contained in the Marburg Collection in *V. natriegens*, we developed a reliable plate reader measurement workflow for reporter experiments and overcame organism-specific challenges, including rapid growth and a high susceptibility towards metabolic burden. By applying this workflow, we generated a comprehensive data set about the quantitative behavior of genetic building blocks in *V. natriegens*. We think that the Marburg Collection as well as the acquired data of the genetic parts will provide valuable resources for the growing *V. natriegens* community.

## 225-MSMV

Recombinant production of prodiginines in engineered *Pseudomonas putida* strains

\*R. Weihmann<sup>1</sup>, F. Kofler<sup>1</sup>, S. Ibrahim<sup>1</sup>, F. Gätgens<sup>1</sup>, J. Gebauer<sup>2</sup>, A. Domröse<sup>1</sup>, S. Thies<sup>1</sup>, S. Kubicki<sup>1</sup>, H. U. C. Brass<sup>2</sup>, A. S. Klein<sup>2</sup>, J. Pietruszka<sup>2,3</sup>, T. Drepper<sup>1</sup>, K. E. Jaeger<sup>3,1</sup>, A. Loeschcke<sup>1</sup>

<sup>1</sup>Institute of Molecular Enzymotechnology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

<sup>2</sup>Institute of Bioorganic Chemistry, Heinrich-Heine University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

<sup>3</sup>Institute of Bio- and Geosciences IBG-1: Biotechnology, Forschungszentrum Jülich GmbH, Jülich, Germany

## Background

High-value compounds such as the bacterial tripyrrolic compound prodiginosin can readily be produced by recombinant biosynthesis in the amenable host *Pseudomonas putida*<sup>1</sup>. As prodiginine variants differ in bioactive properties, it is desirable to make a variety of related structures accessible.

## Methods

We have established the yTRES "gene cluster transplantation tool" which is based on ligase independent cloning to assemble a vector construct carrying a biosynthetic gene cluster of interest and genetic elements that enable the robust conjugational transfer to a bacterial host, and the subsequent insertion in the bacterial chromosome<sup>1</sup>. The yTRES expression tool was re-designed to obtain a modular architecture allowing for flexible cloning and effective expression of natural or customized gene clusters in different modi.

## Results

We combined prodiginine biosynthetic genes from *Serratia marcescens* and *Pseudoalteromonas rubra* for the biosynthesis of different natural prodiginines in *P. putida* KT2440. Here, random genomic integration enabled the identification of naturally highly transcribed chromosomal rDNA operons as suitable regions for gene cluster insertion and expression<sup>2</sup>. Based on this finding, we have generated strains allowing to directly integrate genes into these specific

loci. Furthermore, we assembled distinct pathway parts and achieved the production of precursor molecules like the bipyrrole MBC, which enables creating new-to-nature prodiginine compounds via mutasynthesis<sup>3</sup>. In the course of our studies, we have also demonstrated that the concomitant expression of reporter genes encoding enzymes like  $\beta$ -galactosidase or polyester hydrolase as well as fluorescent proteins proved highly useful to indicate gene cluster transcription levels in different *P. putida* clones.

## Conclusion

Our methodology enables the rapid generation of *P. putida* production strains for the synthesis of valuable compounds such as prodiginines.

<sup>1</sup>Domröse *et al.* 2017, doi:10.1016/j.synbio.2017.11.001

<sup>2</sup>Domröse *et al.* 2019, doi:10.1038/s41598-019-43405-1

<sup>3</sup>Klein *et al.* 2018, doi:10.1002/cbic.201800154

## 226-MSMV

Development of *Vibrio natriegens*, and its prophage-free variant, as a novel host for biotechnology

\*R. Stella<sup>1</sup>, E. Pfeifer<sup>1</sup>, S. Lorke<sup>1</sup>, J. Frunzke<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, IBG-1: Biotechnology, Jülich, Germany

The marine bacterium *Vibrio natriegens* was recently demonstrated to be a promising new host for molecular biology and next generation bioprocesses[1, 2]. *V. natriegens* is a Gram-negative, non-pathogenic slight-halophilic bacterium, with a high nutrient versatility and a reported doubling time of under 10 minutes[2]. However, *V. natriegens* is not an established model organism yet, and work on different aspects is required to foster it into a microbial workhorse. In this work, improvements at the process site and at the microbial site were investigated.

In a first set of experiments, defined growth media were optimized for use in high throughput microtiter plate cultivations and evolutionary experiments. Furthermore, two biosensors, Lrp and LysG, were adapted for expression in *V. natriegens*, to facilitate the detection of positively charged amino acids, L-methionine and branched-chain amino acids. Transcription factor-based biosensors are powerful tools for various applications in biotechnology, examples are to screen and select for cells with increased product formation.

Further experiments focused on the construction of a robust chassis strain. Genome sequencing and qPCR analysis revealed that the first chromosome of *V. natriegens* ATCC 14048 contains two prophage regions (VNP1 and VNP2), which are both still inducible by the DNA-damaging agent mitomycin C and which exhibit spontaneous activation under standard cultivation conditions[3]. A prophage free strain was created, which featured an improved tolerance to DNA-damaging conditions and hypo-osmotic conditions. In a competitive growth experiment, the prophage-free strain outcompeted the wild type. Consequently, this strain is a promising platform for future metabolic engineering strategies.

[1] M. T. Weinstock, E. D. Hesek, C. M. Wilson, D. G. Gibson, *Nat. Methods*, **2016**, 13, 849.

[2] E. Hoffart, S. Grenz, J. Lange, R. Nitschel, F. Müller, A. Schwentner, A. Feith, M. Lenfers-Lücker, R. Takors, B. Blombach, *Appl. Environ. Microbiol.*, **2017**, 83, e01614.

[3] E. Pfeifer, S. Michniewski, C. Gätgens, E. Münch, F. Müller, T. Polen, A. Millard, B. Blombach, J. Frunzke, *Appl. Environ. Microbiol.*, **2019**, 85, e00853.

## 227-SeSiV

### Characterization of a novel Ser/Thr kinase in *Escherichia coli*

\*F. Gratani<sup>1</sup>, T. Englert<sup>1</sup>, M. Semanjski<sup>1</sup>, B. Maček<sup>1</sup>

<sup>1</sup>*Proteome Centre Tübingen, Tübingen, Germany*

Sensing and responding to stimuli drive the regulation of many essential cellular mechanisms. Speed and efficiency of sensing and response systems are the cornerstone of bacterial survival and adaptability. In order to respond to signals, bacteria adopted different strategies, from synthesis of messengers to post-translational modification (PTM). Phosphorylation is the most studied PTM and is the pivotal strategy used to translate different signals into physiological responses. In eukaryotes, Ser/Thr/Tyr kinase have a major role in this signal transduction due to the wide number and types of proteins targeted; although widespread in bacteria, this type of PTM is still relatively underexplored. Recently, we identified the YjjJ protein as a novel target of the persistence-related Ser/Thr kinase HipA7. Sequence analysis indicate that YjjJ is a putative kinase belonging to the HipA-like family, with conserved residues for phosphorylation activity. Here we aimed to characterize the kinase activity of YjjJ, identify its targets and assess its effect on *E.coli* physiology. We first tested the impact of YjjJ on bacterial growth and its potential role in antibiotic tolerance. Using liquid chromatography coupled with mass-spectrometry, we performed shotgun phosphoproteomics and analyzed the impact of YjjJ overexpression on the proteome and phosphoproteome. We confirmed phosphoproteomic results via *in vitro* kinase and complementation assay. Our phosphoproteomic results revealed significant changes in phosphorylation levels on several proteins and pointed to GltX as the main YjjJ target. We then confirmed YjjJ substrates via *in vitro* assays coupled with complementation tests. We demonstrated that YjjJ activity is toxic for the cell and at high levels leads to cell death. Here we show for the first time that YjjJ is a Ser/Thr kinase that phosphorylates GltX (also a HipA target) and is toxic for the cell. Despite its high similarities with HipA, YjjJ does not seem to be involved in persistence, leaving the role of this kinase still unknown.

## 228-SeSiV

Transcription of the PCE regulon in organohalide-respiring *Sulfurospirillum* species is controlled by a two-component system.

J. Esken<sup>1</sup>, \*T. Schubert<sup>1</sup>

<sup>1</sup>*Friedrich-Schiller-Universität Jena, Institute of Microbiology, Jena, Germany*

The genetic inventory required for the tetrachloroethene (PCE) respiration performed by *Sulfurospirillum halorespirans* or *Sulfurospirillum multivorans* (phylum: Campylobacterota) is combined in a single genetic locus comprising eight transcriptional units. The eight operons encode for the norcobamide-containing PCE reductive dehalogenase, for the norcobamide cofactor biosynthetic machinery, for components of the PCE respiratory chain, and for a pair of two-component systems (TCSs). Gene

transcription is readily inducible by PCE within one generation, but ceases in the absence of PCE in a long-term downregulation (>100 generations). In previous genomic, proteomic, and transcriptomic analyses evidence was found that one out of the two TCSs is involved in PCE sensing and transcription initiation.

Here, we raised the question for the presence of an operator sequence recognized by the regulator and common to all promoters of the organohalide respiration gene region. In order to analyze the interaction between the response regulator protein (RR2) and the promoter sequences, purified RR2 was tested for binding different intergenic regions in electrophoretic mobility shift assays (EMSA). For the identification of the binding sites, libraries of DNA fragments covering short sections of the intergenic regions were screened. By using this approach, the interaction of RR2 with all eight promoter sequences of the PCE regulon was observed. In addition, a direct repeat (consensus CTATW separated by 17 bp) was identified as the regulator protein's binding site either located close to the -35 box or further upstream. Finally, based on the evaluation of the transcript levels in combination with the results of the EMSA a model for the PCE-sensing regulatory network in dehalogenating *Sulfurospirillum* species was derived in which RR2 acts as class I and class II transcriptional activator.

## 229-SeSiV

Control of the bifunctional O<sub>2</sub>-sensor kinase NreB of *Staphylococcus carnosus* by the nitrate sensor NreA

\*R. Klein<sup>1</sup>, A. K. Kretschmar<sup>1</sup>, G. Uuden<sup>1</sup>

<sup>1</sup>*Johannes Gutenberg-University Mainz, Institute of Molecular Physiology, Mainz, Germany*

R. Klein<sup>\*1</sup>, A. Kretschmar<sup>1</sup> and G. Uuden<sup>1</sup>

<sup>1</sup>Microbiology and Wine Research, Institute for Molecular Physiology, Johannes Gutenberg-University, Mainz, Germany

The NreABC two-component system induces expression of the nitrate reductase (*narGHJ* genes) of *Staphylococci* in the presence of nitrate and anaerobic conditions<sup>1</sup>. NreB represents the oxygen sensor while NreC acts as the response regulator<sup>2</sup>. Under anaerobic conditions the sensor kinase NreB is autophosphorylated and phosphorylates the response regulator NreC. NreA represents a nitrate sensor<sup>3</sup> which controls NreB phosphorylation in response to nitrate availability<sup>1</sup>, resulting in a nitrate/oxygen co-sensing unit.

NreB contains a DxxxQ phosphatase motive of HisKA\_3 type histidine kinases, suggesting location of the phosphatase site of the NreB-NreC system in NreB. NreC exhibited no capacity for auto-dephosphorylation. NreB was able to dephosphorylate NreC-phosphate, but required the joint presence of NreA for dephosphorylation. The NreA regulated phosphatase activity of NreB was lost in the presence of nitrate. NreB mutants of the DxxxQ motive were not able to dephosphorylate NreC-phosphate. Therefore, NreB represents a bifunctional sensor histidine kinase which regulates the phosphorylation state of NreC. The switch in NreB function is controlled by NreA by inhibiting NreB autophosphorylation and stimulating the phosphatase in the free state (apoNreA) or by stimulating NreB autophosphorylation and inhibiting the phosphatase in the nitrate bound form (NreA-nitrate complex).

[1] Nilkens *et al.* (2014); *Molecular microbiology* 91 (2): 381–393

[2] Müllner *et al.* (2008); *Biochemistry* 47 (52): 13921–13932

[3] Niemann *et al.* (2014); *Journal of Molecular Biology* 426 (7): 1539–1553

### 230-SeSiV

#### Phenotypical and functional characterization of the bacterial phytochrome *PaBphP* of *Pseudomonas aeruginosa*

\*C. Huber<sup>1</sup>, J. Pielage<sup>1</sup>, N. Frankenberg-Dinkel<sup>1</sup>, K. Bilici<sup>1</sup>, L. Denig<sup>1</sup>

<sup>1</sup>Technische Universität Kaiserslautern, Institut für Mikrobiologie, Kaiserslautern, Germany

The opportunistic pathogen *Pseudomonas aeruginosa* encodes a red and far-red light sensing bacteriophytochrome (*PaBphP*). Phytochromes are photoreceptors found in plants, algae, fungi and bacteria that regulate a variety of vital processes in response to environmental light conditions and have also been linked to virulence in plant pathogens. These photochromic biliproteins form dimers with each monomer possessing a covalently linked chromophore, biliverdin IX<sub>a</sub>, and there are two spectrally distinct forms: a red light-absorbing Pr and a far-red light-absorbing Pfr form. In plants, it has been shown that the phototransformation between the two forms induces a signaling network, which includes interaction with a wide array of signaling partners and transcriptional regulation of various photoresponsive genes. However, the role of the bacteriophytochrome in *P. aeruginosa* is still unknown and the biochemical mechanisms of phytochrome function have not been fully elucidated.

In previous studies we established that the genes encoding the two necessary bacteriophytochrome components of *P. aeruginosa* BphO, a chromophore-producing heme oxygenase, and BphP, the apo-phytochrome, are co-transcribed in a bicistronic operon. Transcription was shown to be induced in the stationary phase in a cell-density-dependent manner and to be dependent on the alternative sigma factor RpoS. We furthermore demonstrated autophosphorylation of *PaBphP*, confirming its role as a sensor kinase and a postulated sensor of a two-component regulatory system, but the corresponding response regulator is still unknown.

In order to elucidate the biological function of *PaBphP* we constructed a number of *Pseudomonas* mutant strains of *bphP*, which we employed for phenotypical characterizations in red and far-red illumination. By applying homologously overexpressed *bphP* for cross-linking experiments and proteomics we hope to gain insight into the downstream signaling pathway of *PaBphP* and advance our understanding of the role of a light sensor in *P. aeruginosa*.

### 231-SeSiV

#### Control of light-dependent type 4 pilus activity during cyanobacterial phototaxis

\*N. Schuergers<sup>1</sup>, Y. Han<sup>1</sup>, A. Jakob<sup>1</sup>, A. Wilde<sup>1</sup>

<sup>1</sup>Albert-Ludwigs-Universität Freiburg, Institut für Biologie III - Molekulare Genetik, Freiburg, Germany

Many prokaryotes show complex behaviors that require the intricate spatial and temporal organization of cellular protein machineries, leading to asymmetrical protein distribution and cell polarity. One such behavior is the Type 4 pili (TFP)-mediated phototactic motility of cyanobacteria which relies on the dynamic localization of the motility motors in response to light[1]. Although we showed that individual *Synechocystis* cells directly sense the direction of incoming light, we do not

understand how the light signal leads to the polar activity of the motility apparatus[2]. However, experimental and genetic evidence suggest that photoreceptors, chemosensory systems and PatA-type response regulators with a PATAN-REC domain architecture are key players in establishing light-induced cell polarity during phototactic movement. We want to unravel how these signaling complexes communicate with the TFP to control localized pilus assembly.

Employing fluorescence imaging and interaction studies we found that PatA-type regulators can directly interact with components of the motility apparatus (PilB/PilC) at the cytoplasmic membrane[3]. Intriguingly, the PATAN-domain is sufficient for binding to the pilus machinery while the role of the REC domain is probably limited to a regulatory function. PATAN-domain proteins, which so far have not been functionally characterized, are commonly found in environmental bacteria like *Myxococcus* or *Geobacter* species that show complex spatial organization and it seems likely that this domain is involved in the regulation of spatiotemporal dynamics of different cellular processes.

1. Schuergers, N., Nürnberg, D. J., Wallner, T., Mullineaux, C. W. & Wilde, A. PilB localization correlates with the direction of twitching motility in the cyanobacterium *Synechocystis* sp. PCC 6803. *Microbiology* 161, 960–966 (2015).
2. Schuergers, N. *et al.* Cyanobacteria use micro-optics to sense light direction. *Elife* 5, e12620 (2016).
3. Jakob, A. *et al.* The (PATAN)-CheY-like Response Regulator PixE interacts with the Motor ATPase PilB1 to control Negative Phototaxis in the Cyanobacterium *Synechocystis* sp. PCC 6803. *Plant and Cell Physiology*, pcz194.

### 232-SeSiV

#### Regulation of motility in *Vibrio cholerae* by RNA-binding protein ProQ

\*D. Devlitsarov<sup>1</sup>, K. Papenfort<sup>2</sup>

<sup>1</sup>LMU, Jena, Germany

<sup>2</sup> Friedrich-Schiller-Universität, Jena, Germany

Bacterial small regulatory RNAs (sRNAs) modulate gene expression via limited base-pairing with target mRNAs. RNA-binding proteins are often required to stabilize the sRNAs and to facilitate base-pairing of the sRNAs with their targets. In many cases, this base-pairing interaction is mediated by the RNA chaperone, Hfq. However, many sRNAs are not recognized by Hfq, and not all bacteria encode Hfq homologues. This raises the possibility of other RNA chaperones facilitating the interaction between regulatory sRNAs and their targets. ProQ is an RNA-binding protein that has recently been discovered in *Salmonella enterica*. ProQ has been shown to bind over 400 cellular transcripts, however, little is known about the mechanism by which ProQ recognizes its ligand sRNAs and their target mRNAs, and how it mediates their interaction. In this work, we determined the set of ProQ binding partners in *V. cholerae* and discovered that ProQ regulates swimming motility. We discovered dozens of sRNAs and hundreds of mRNAs binding ProQ in *V. cholerae* at low and high cell densities. One sRNA, called FlaX, was efficiently co-immunoprecipitated with ProQ and we show that this sRNA is produced from the 3' UTR of *flaA*, a gene encoding a core flagellin protein required for motility. Deletion of *flaX* resulted in reduced motility of *V. cholerae*, whereas over-expressing FlaX had the reverse effect. Deletion and overexpression of *proQ* showed the same trend. Using a transcriptomic approach, we identified the *flaB* mRNA, encoding a

secondary flagellin, as the chief target of FlaX and showed that regulation depends on direct base-pairing of the two transcripts. Together, our data indicate a global role for ProQ in gene regulation of *V. cholerae* and suggest that cell motility is controlled at the post-transcriptional level by a ProQ-associated sRNA.

### 233-SeSiV

How the most abundant ions become toxic to *Bacillus subtilis*

\*L. Krüger<sup>1</sup>, C. Herzberg<sup>1</sup>, J. Stülke<sup>1</sup>

<sup>1</sup>Georg-August Universität Göttingen, Institut für Mikrobiologie und Genetik, Allgemeine Mikrobiologie, Göttingen, Germany

Potassium and glutamate are by far the most abundant ions in any living cell. Thus, the cytoplasm can be regarded as a solution of potassium and glutamate [1]. Potassium is essential for many cellular processes. Its positive charge buffers the negative charges of both the DNA backbone and its counterion glutamate. Glutamate serves as amino group donor for amino acid synthesis. In order to meet the cellular demand for these two ions, efficient regulatory systems are indispensable. Cyclic di-adenosine monophosphate (c-di-AMP) is a second messenger used by the Gram-positive bacterium *Bacillus subtilis* and other firmicutes. C-di-AMP controls the expression and activity of the potassium transporters [2]. Glutamate acquisition can be achieved via transport and synthesis of the amino acid. We observed that a c-di-AMP-free strain was not viable at increased potassium concentrations [2] or in the presence of glutamate. However, we were able to isolate suppressor mutants that allow growth of a c-di-AMP-free strain under both toxic conditions. Characterization of glutamate suppressors revealed that they contain pairs of mutations affecting glutamate and potassium homeostasis. Several independent mutations affected a novel glutamate transporter. Moreover, we found evidence that glutamate directly affects potassium uptake. With this, we can add another layer to the complexity of the essentiality of c-di-AMP in *B. subtilis*.

### References

[1] Bennett *et al.*, 2009 *Nat Chem Biol.*, **5**(8): 593–599.

[2] Gundlach *et al.*, 2017 *Sci. Signal.*, **10**, eaal3011.

### 234-SeSiV

Staying or leaving: c-di-GMP regulated cell behavior in probiotic *E. coli* Nissle 1917

\*A. M. Cimdins-Ahne<sup>1</sup>, V. Mete<sup>1</sup>, M. Kiel<sup>1</sup>, H. Bähre<sup>2</sup>, V. Kaefer<sup>2</sup>, U. Dobrindt<sup>1</sup>

<sup>1</sup>University of Muenster, Institute of Hygiene, Muenster, Germany

<sup>2</sup>Hannover Medical School, Research Core Unit Metabolomics, Hannover, Germany

### INTRODUCTION

*Escherichia coli* Nissle 1917 (EcN) is an established probiotic, used in treatment of gastrointestinal infection and inflammation. *In vivo* biofilm formation is likely to promote EcN retention in the host, while motility aids in colonization. When grown on low salt agar plates at ambient temperature, *E. coli* strains feature a biofilm matrix built up of curli fimbriae and cellulose. At 37°C, the EcN biofilm matrix consists of cellulose only, while standard *E. coli* do not form biofilms. Of

note, cellulose synthesis is independent of the transcriptional activator CsgD, otherwise considered as the master biofilm regulator. In EcN, only curli genes are expressed CsgD-dependently. Moreover, c-di-GMP necessary for allosteric activation of the cellulose synthase is not provided by AdrA (DgcC), but is from an unknown source.

### OBJECTIVES

We want to understand the regulation behind the decision for motility or biofilm formation at 37°C in EcN and to identify the source of c-di-GMP required for activation of cellulose synthesis.

### MATERIALS AND METHODS

Genomes of EcN strains were analyzed for variations among the c-di-GMP turnover proteins and biofilm related genes. Phenotypical analyses included motility assays in low agar plates, biofilm assay, and gene expression analyses. Intracellular c-di-GMP levels were determined by quantitative LC-MS/MS.

### RESULTS

Genomic analysis of EcN wild type and spontaneously occurred EcN biofilm-defective mutants identified the diguanylate cyclase YegE (DgcE) as the likely c-di-GMP provider for activation of cellulose synthesis. Indeed, *yegE::cat* deletion strains were likewise incapable of forming biofilm. Additionally, these strains were highly motile. Analysis of nucleotide extracts demonstrated a low amount of c-di-GMP. Increased flagella expression was excluded by qRT-PCR testing for selected flagellar genes and immunodetection of flagellin subunits.

### CONCLUSION

YegE (DgcE) is known to be part of the *csgD* regulation cascade in model *E. coli* strains. Surprisingly, unlike *E. coli* K-12  $\Delta yegE$ , EcN  $\Delta yegE$  is incapable of forming biofilm. Thus, YegE, and not CsgD, is the main regulator for the motility-sessility switch in probiotic EcN.

### 235-EPV

Molecular characterization of *Giardia duodenalis*

A. Woschke<sup>1</sup>, M. Faber<sup>2</sup>, K. Stark<sup>2</sup>, M. Holtfreter<sup>3</sup>, T. Regnath<sup>4</sup>, I. Sobottka<sup>5</sup>, A. Diefenbach<sup>6</sup>, R. Ignatius<sup>6,7</sup>, A. Aebischer<sup>1</sup>, \*C. Klotz<sup>1</sup>

<sup>1</sup>Robert Koch-Institut, FG16 Parasitology, Berlin, Germany

<sup>2</sup>Robert Koch-Institut, FG35, Berlin, Germany

<sup>3</sup>Universitätsklinikum Düsseldorf, Gastroenterologie, Hepatologie und Infektiologie, Düsseldorf, Germany

<sup>4</sup>Laboratory Enders and Partners, Stuttgart, Germany

<sup>5</sup>LADR GmbH, Geesthacht, Germany

<sup>6</sup>Charité-Universitätsmedizin Berlin, Institute of Microbiology and Infection Immunology, Berlin, Germany

<sup>7</sup>Labor 28 GmbH, Berlin, Germany

Introduction and objectives: The eukaryotic parasite *Giardia duodenalis* causes diarrheal disease in humans worldwide and is an important public health concern. Eight distinct genetic groups (referred to as assemblage A-H) are known, of which only assemblage A and B appear to be pathogenic to humans and animals. This pilot project aimed at applying typing methods in order to identify and discriminate assemblages and sub-assemblages of *G. duodenalis* at the isolate level, an approach that is currently not standardized and routinely applied. Challenges for isolate identification include the tetraploid nature of the parasite, occurrence of



mixed infections and that these organisms are not culturable at a routine level.

**Materials and methods:** DNA of a collection of *G. duodenalis* positive human stool samples were extracted and the underlying assemblage types assigned by qPCR. Subsequently, *G. duodenalis* isolates were classified by multi locus sequence genotyping (MLST) schemes in order to test the typing resolution of these methods.

**Results:** More than 120 *G. duodenalis* positive samples were successfully typed at one or more loci. Approximately 60% belonged to assemblage B, 20% to assemblage A and 20% were mixed with A and B. A test typing data set containing sequences from isolates longitudinally sampled from individual patients and from isolates obtained from unrelated travel associated and autochthonous cases revealed that for assemblage A and B different MLST schemes are necessary to trace linked infections. It was also found that mixed infections interfere with successful isolate tracing.

**Conclusion:** Established MLST schemes may be of help to trace linked infections of *G. duodenalis* detected in fecal human samples. However, further improvements are necessary to identify and adequately type isolates in samples with (intra-assemblage) mixed infections.

---

### 236-EPV

A novel protein essential for unconventional secretion of chitinase Cts1 in *Ustilago maydis*

\*M. Reindl<sup>1</sup>, J. Stock<sup>1</sup>, K. Hussnaetter<sup>1</sup>, S. Wolf<sup>1,2</sup>, M. Feldbrügge<sup>1</sup>, K. Schipper<sup>1,2</sup>

<sup>1</sup>Heinrich Heine University Duesseldorf, Institute for Microbiology, Duesseldorf, Germany

<sup>2</sup>Heinrich Heine University Duesseldorf, Duesseldorf, Germany

The chitinase Cts1 of the smut fungus *U. maydis* is secreted by a novel unconventional lock-type mechanism into the extracellular environment (1). During cytokinesis of yeast-like cells Cts1 accumulates in the small compartment, the fragmentation zone, that is formed between dividing mother and daughter cells (2). Together with a second chitinase, Cts1 supports the separation of the two cells by degrading the connecting cell wall. Up to date the exact mechanism of the transport into the fragmentation zone is poorly understood. In a UV mutagenesis screen for cells deficient in Cts1 secretion the gene *jps1* was identified. Encoding a protein of 60 kDa and lacking characterized orthologues, Jps1 (Jammed in Protein Secretion Screen 1) strongly influences proper localization and secretion of Cts1 (Reindl and Stock, unpublished). Localisation studies showed that Jps1, like Cts1, localises to the fragmentation zone indicating interaction of the two proteins. Interestingly, localisation of Cts1 is dependent on Jps1 but not vice versa. A putative interaction was further supported by yeast two-hybrid experiments. Analysing truncated versions of Jps1 allowed to identify a 60 amino acid region close to its N-terminus which is important for localisation and secretion of Cts1 termed Cts1 localisation domain (CLD). The CLD is rich in basic amino acids and thus might also mediate lipid binding of Jps1. In line with that, lipid interaction studies using PIP strips indicated binding of Jps1 to different PtdInsPs which was abolished in absence of the CLD. Currently the lipid binding profile of Jps1 is under further investigation using giant unilamellar vesicles (GUV). We hypothesize that Jps1 might act as membrane anchoring factor for unconventional Cts1 secretion.

(1) Aschenbroich et al., (2018): The germinal centre kinase Don3 is crucial for unconventional secretion of chitinase Cts1 in *Ustilago maydis*. BBA- Proteins and Proteomics

(2) Langner et al., (2015): Chitinases are essential for cell separation in *Ustilago maydis*. Eukaryotic Cell, 14, 846-857

---

### 237-EPV

Human serum albumin differentially modulates *Candida*-induced damage of vaginal epithelium on a species level

\*M. Pekmezovic<sup>1</sup>, A. K. Kaune<sup>2,3</sup>, S. Austermeier<sup>2</sup>, S. Mogavero<sup>2</sup>, M. Gresnigt<sup>2</sup>, B. Hube<sup>2,4</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Microbial Pathogenicity Mechanisms, Jena, Germany

<sup>2</sup>Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knoell-Institute, Microbial Pathogenicity Mechanisms, Jena, Germany

<sup>3</sup>MRC Centre for Medical Mycology at University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, United Kingdom

<sup>4</sup>Friedrich Schiller University, Jena, Germany

Vulvovaginal candidiasis (VVC) affects 70-75% of women at least once in their lifetime. Although *Candida albicans* predominantly causes VVC, the prevalence of other species, like *C. glabrata*, has increased. Despite the same genus name, these species differ not only phylogenetically, but also in terms of pathogenicity and their interactions with the host. *C. albicans* can grow in both yeast and hyphal form, causes a strong immune response and secretes the peptide toxin candidalysin (CaL) that causes epithelial damage. On the other hand, *C. glabrata* normally exists only as yeast, elicits a weak immune response, and has pathogenicity mechanisms that remain to be elucidated.

Human serum albumin (HSA) is the most abundant protein in cervico-vaginal secretions and previous reports indicated that its concentration changes during infection. Therefore, we aimed to investigate the influence of HSA on the outcome of *Candida* infection of vaginal epithelial cells.

Using an *in vitro* vaginal infection model, we assessed the adhesion, invasion, and damage properties of *C. albicans* and *C. glabrata* in the presence of HSA. Additionally, we investigated the influence of HSA on fungal growth, morphology, iron acquisition, and CaL-induced damage.

We observed that HSA reduced the adhesion, invasion, and damage capacity of *C. albicans*. Although HSA was growth-promoting, it increased pseudohyphae formation and neutralized CaL-induced damage. On the contrary, adhesion and epithelial damage by *C. glabrata* were significantly increased in the presence of HSA. *C. glabrata* growth was also increased, but only in the presence of host cells. Finally, we observed that HSA influenced iron availability to *C. glabrata* during infection, thus providing the fungus with a mechanism to avoid host iron restriction.

Collectively, these results show that HSA influences the VVC outcome differentially in species-dependent manner. Since both species are predominantly commensals on vaginal mucosa, we hypothesize that varying concentration of albumin may influence the commensal-to-pathogen shift and determine which *Candida* species would have more favorable conditions for causing the VVC.

---

### 238-EPV

Conidial melanin of the human pathogenic fungus *Aspergillus fumigatus* disrupts cell autonomous defenses in amoebae

\*I. Ferling<sup>1,2</sup>, J. D. Dunn<sup>3</sup>, A. Ferling<sup>4</sup>, T. Soldati<sup>3</sup>, F. Hillmann<sup>1</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute (HKI), Evolution of Microbial Interactions, Jena, Germany

<sup>2</sup>Friedrich Schiller University, Jena, Germany

<sup>3</sup>University of Geneva, Department of Biochemistry, Geneva, Switzerland

<sup>4</sup>Technische Schule Heid-Tech, Heidenheim, Germany

The human pathogenic fungus *Aspergillus fumigatus* is a ubiquitous saprophyte that causes fatal infections in immunocompromised individuals. Following inhalation, conidia are ingested by innate immune cells and can arrest phagolysosome maturation. How such general virulence traits could have been selected for in natural environments is unknown. Here, we used the model amoeba *Dictyostelium discoideum* to follow the antagonistic interaction of *A. fumigatus* conidia with environmental phagocytes in real-time. We found that conidia covered with the green pigment 1,8-dihydroxynaphthalene-(DHN)-melanin were internalized at far lower rates when compared to those lacking the pigment, despite high rates of initial attachment. Immediately after uptake of the fungal conidia, nascent phagosomes were formed through sequential membrane fusion and fission events. Using single-cell assays supported by a computational model integrating the differential dynamics of internalization and phagolysosome maturation, we could show that acidification of phagolysosomes was transient and was followed by neutralization and, finally, exocytosis of the conidium. For unpigmented conidia, the cycle was completed in less than one hour, while the process was delayed for conidia covered with DHN-melanin. At later stages of infection, damage to infected phagocytes triggered the ESCRT membrane repair machinery, whose recruitment was also attenuated by DHN-melanin, favoring prolonged persistence and the establishment of an intracellular germination replication niche in this environmental phagocyte. Increased exposure of DHN-melanin on the conidial surface also improved fungal survival when confronted with the fungivorous predator *Protostelium aurantium*, demonstrating its universal anti-phagocytic properties.

## 239-EPV

The gut commensal *Bacteroides vulgatus* mpk reduces *Candida albicans* pathogenicity *in vitro* and *in vivo*

\*M. J. Niemiec<sup>1,2</sup>, I. Auge<sup>1</sup>, P. van Leeuwen<sup>1</sup>, F. Gorki<sup>1</sup>, A. Steimle<sup>3,4</sup>, M. Brüggmann<sup>1</sup>, J. S. Frick<sup>4</sup>, I. D. Jacobsen<sup>1,2,5</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection - Hans Knöll Institute, Research Group Microbial Immunology, Jena, Germany

<sup>2</sup>Center for Sepsis Control and Care, Jena, Germany

<sup>3</sup>Luxembourg Institute of Health, Luxembourg, Luxembourg

<sup>4</sup>University of Tübingen, Tübingen, Germany

<sup>5</sup>Friedrich Schiller University, Jena, Germany

The human gastro intestinal tract is densely colonized by a variety of commensal bacteria and fungi, among them the commonly found yeast *Candida albicans*. Mostly harmless, this opportunistic pathogen can cause disease, ranging from superficial infections to sepsis, if the local equilibrium of host, bacteria and *Candida* is disturbed. Commensal bacteria play an important role in this equilibrium as they protect the host directly or indirectly from pathogen-mediated infection or inflammation. *Bacteroides vulgatus* mpk is well-known for its positive immunomodulatory properties in mouse models of inflammatory bowel disease. Yet, its potential interplay with the commensal gut microbiota is unknown.

In this study we aimed to test the anaerobic commensal *B. vulgatus* mpk for its protective properties towards *C. albicans* pathogenicity *in vitro* and *in vivo*.

For this, we first cocultivated *C. albicans* and *B. vulgatus* mpk for several days under normoxic and hypoxic conditions. Here, we detected prolonged survival of *Bacteroides* upon presence of *C. albicans*.

Secondly, we infected enterocytes *in vitro* with *B. vulgatus* mpk prior to *C. albicans* infection and compared cell damage to *Candida*-only infections. While *B. vulgatus* mpk reduced enterocyte damage by *C. albicans* if added prior to or simultaneously to the host cells, delayed addition of *B. vulgatus* mpk could not mediate rescue of the enterocytes.

Finally, we co-challenged *Caenorhabditis elegans* with *Candida* and *Bacteroides* and could detect prolonged survival compared to *Candida*-only infected nemaodes.

Currently, experiments identifying key mediators during *B. vulgatus* mpk-mediated host cell protection are ongoing. With this we aim to foster further understanding of the vital host-bacteria-fungi triangle.

## 240-FMV

**Combining isothermal microcalorimetry and microbiological analytics for fast and reliable detection of microbial contaminations**

\*C. Fricke<sup>1</sup>, H. Harms<sup>1</sup>, T. Maskow<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research, Department Environmental Microbiology, Leipzig, Germany

## Introduction

One of the most important challenges in quality control is the fast detection and quantification of microbial contaminations in water, food, and pharmaceuticals. Culture-based techniques like visual counting of colony-forming units (CFUs) are still considered as the standard in microbiological analytics. The key advantages are ease of operation, simple data evaluation and low costs. However, there are two main serious weaknesses, namely slow detection and subjective decisions when quantifying bacterial contaminations. To use the advantages of classical cultivation while at the same time reducing the weaknesses, isothermal microcalorimetry (IMC) can be used as a culture-based detection method in the area of microbiological analytics. We propose to replace the eye with a much more sensitive detector (Peltier-elements) in IMC since they can detect the smallest traces of heat, which occurred as a by-product of microbial growth and the signal is directly correlated with the numbers of active bacteria.

## Methods

In our study, we investigated the practicability of cultivation techniques in the context of IMC measurements using *Lactobacillus plantarum* 17-5 and *Pseudomonas putida* mt-2 KT2440 as model bacteria for an anaerobic and aerobic system, respectively. For this reason, we have monitored bacterial growth on solid and liquid culture (SC and LC) media using IMC. We compared the results by visual inspection of CFU-counting and turbidity measurements.

## Results

Our results demonstrate that IMC monitoring provides reduced detection time in both SC and LC. Also, the use of

classical enrichment methods such as membrane filtration offers another possibility to shorten the detection time. By using a simple physical model, we can estimate the number of contaminating bacteria using the first appearance of the thermal signal regardless of the cultivation technique used.

## Conclusion

Our study provides valuable information for the development of IMC as an early warning system for bacterial contaminations and might be incorporated into standardized protocols in microbiological analytics.

## Reference

Fricke, C., Harms, H., and Maskow, T. (2019). Front Microbiol 10.

### 241-FMV

Repeated microwave sanitization adversely alters the genetic potential of the kitchen sponge microbiome

\*S. Jacksch<sup>1</sup>, J. Thota<sup>1</sup>, M. Egert<sup>1</sup>

<sup>1</sup>Institute of Precision Medicine / Furtwangen University, Villingen-Schwenningen, Germany

**Background:** Used kitchen sponges massively absorb and spread microorganisms, leading to microbial contaminations of kitchen surfaces and food [1,2]. Quick microwaving is a widely used technique to achieve a short-term reduction of the microbial load in kitchen sponges. However, long-term effects of microwaving on microbiome structure and functionality are largely unknown, although it has been speculated that sanitizing and cleaning treatments might select for potential pathogens and/or malodor producers [1].

**Methods:** Newly purchased domestic polyurethane kitchen sponges were distributed to 20 study participants. Each participant was instructed to use the sponge under normal household conditions for a period of ~ 4 weeks. Ten participants were asked to clean their sponges regularly by standardized microwaving, while the remaining ten sponges remained uncleaned. After DNA extraction and Illumina MiSeq-based shotgun sequencing, all data were uploaded to MG-RAST for taxonomic and functional annotation.

**Results:** Sequence evaluation indicated a relative dominance of the domain of *Bacteria* (97%). The remaining sequences were assigned to viruses, eukaryotes and archaea. A comparison of sanitized and untreated kitchen sponges indicated a trend towards a reduced microbial diversity in regularly microwaved sponges. Microwaved sponge samples also showed higher shares of bacterial genera with a pathogenic potential, such as *Acinetobacter*. Furthermore, regular microwaving significantly influenced the functional properties encoded in the kitchen sponge community, such as sulfur and cell wall/capsule metabolism.

**Conclusions:** Microwave sanitization alters community composition and metabolic properties of microbial communities in used kitchen sponges. Clearly, further work is needed to elucidate in more detail what this means in terms of kitchen hygiene. Nevertheless, our data seem to confirm previous assumptions [1] that typical domestic sanitization strategies might alter domestic microbial communities in way that is rather negative for human health and well-being.

[1] Cardinale et al. (2017) Sci Rep 7(1): 5791, [2] Sharma et al. (2009) Food Control 20(3): 310–313

### 242-FMV

Effect of wash water additives on the microbial and sensory quality of curly parsley

S. Nübling<sup>1</sup>, F. Hägele<sup>2</sup>, R. M. Schweiggert<sup>2</sup>, R. Carle<sup>2,3</sup>, H. Schmidt<sup>1</sup>, \*A. Weiß<sup>1</sup>

<sup>1</sup>University of Hohenheim, Department of Food Microbiology and Hygiene, Stuttgart, Germany

<sup>2</sup>University of Hohenheim, Chair of Plant Foodstuff Technology and Analysis, Stuttgart, Germany

<sup>3</sup>King Abdulaziz University, Biological Science Department, Jeddah, Saudi Arabia

**Introduction:** Curly parsley (*Petroselinum crispum* var. *crispum*) is a widely used culinary herb and is mainly consumed raw. Due to its cultivation conditions and its leaf structure it contains high counts of autochthonous microbiota, which may include pathogens. The washing of leaves prior to freezing is the only step during processing for the reduction of the viable counts. Globally, water supplemented with chlorine is applied, though this might result in the formation of off-flavors and carcinogenic halogenated by-products such as trihalomethanes and chloramines.

**Objectives:** The aim of this study was to evaluate the impact of N<sup>α</sup>-lauroyl-L-arginine ethyl ester (LAE) and lactic acid as safe alternative wash water additives in comparison to chlorine in curly parsley processing on pre-industrial scale and to assess selected quality parameters throughout deep-frozen storage.

**Materials & Methods:** Curly parsley was washed in cold (6°C) or warm tap water (45°C), as well as in cold tap water containing 100 mg/L LAE, 4 g/L lactic acid or 200 mg/L chlorine. Parsley without washing was used as a control. Total aerobic mesophilic bacterial viable counts (Standard I agar), *Pseudomonas* spp. (Glutamate starch phenol red agar) and *Enterobacteriaceae* (Violet red bile dextrose agar) viable counts were determined of fresh products and after deep-frozen storage for up to nine months. Dry matter contents, chlorophylls, β-carotene levels, as well as nitrate and nitrite contents were determined throughout the storage. After six months, selected sensory quality attributes were evaluated.

**Results:** LAE reduced total viable counts, *Pseudomonas* spp., and *Enterobacteriaceae* by more than 2.0 log<sub>10</sub> cfu/g, while lactic acid was effective against *Enterobacteriaceae*. Chlorophylls and β-carotene were best retained in parsley washed with LAE, while insignificant differences for nitrate contents and lipoxigenase activities between the different treatments were found. Flavor was best for lactic acid treated parsley.

**Conclusion:** LAE and lactic acid showed equal performance to chlorine in decreasing viable counts and are therefore a safe alternative for curly parsley processing.

### 243-FMV

Time-dependent variation of biofilm community composition on stainless steel surfaces in milking machine

\*M. Weber<sup>1</sup>, A. Lipski<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, IEL - Abteilung Lebensmittelmikrobiologie und -hygiene, Bonn, Germany

Microbial biofilms in milking machines pose a massive threat to milk quality and safety as well as to udder health. Elevated production of heat resistant lipases and proteases in milking

machine biofilms can adversely affect milk quality, even after pasteurization.

As the risk potential of milking machine biofilms is strongly dependent on the community composition of its inhabitants, we aimed to retrace the process of biofilm formation on stainless steel coupons exposed in a milking machine during ongoing operation. We examined exposition time-dependent variations in surface colonization and community composition of biofilms by a combination of culture-dependent and -independent methods. These included cultivation on different selective and non-selective agar media as well as direct DNA-extraction for clone library generation and fluorescence-*in-situ*-hybridization (FISH). Isolates were characterized for biofilm formation on various surface materials and under different environmental conditions relevant to the dairy industry. This also includes analyses of combinations of isolates to detect cooperative or synergistic interactions regarding spoilage potential and biofilm formation.

In contrast to the static character of swab samplings, this study is the first one to characterize the time-dependent dynamics of milking machine biofilms under real operating conditions. This approach also involves the analysis of mixed cultures, which is more representative for the natural ecology of dairy biofilms.

---

## 244-FMV

Revaluation of decontamination strategies against *Campylobacter jejuni* and ESBL-producing *Escherichia coli* on chicken skin by viability qPCR

\*I. F. Wulsten<sup>1</sup>, M. Thieck<sup>1</sup>, A. Göhler<sup>2</sup>, E. Schuh<sup>2</sup>, K. Stingl<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment (BfR), National Reference Laboratory for *Campylobacter*, Department of Biological Safety, Berlin, Germany

<sup>2</sup>German Federal Institute for Risk Assessment (BfR), National Reference Laboratory for *Escherichia coli* including verotoxin-producing *E. coli*, Department of Biological Safety, Berlin, Germany

### 1. Introduction

*Campylobacter* and *Escherichia coli* are classical fecal contaminants along the poultry production chain with serious implications for human health.

### 2. Objectives

Viability of bacteria is commonly quantified by their capacity to form a colony on an agar plate (CFU). Upon stress exposure bacteria might cease cell division while potentially remaining infective. We developed a viability real-time PCR (qPCR) for the culture-independent quantification of living *E. coli*. The novel qPCR was applied in combination with our previously developed viability qPCR for thermophilic *Campylobacter* to reevaluate decontamination strategies based on intact and putatively infectious units (IPIU).

### 3. Methods

We reevaluated decontamination in a practice-oriented chicken skin model with dual spiking of an ESBL-producing *E. coli* and a *C. jejuni* strain. We applied lactic acid (LA, 1% and 5%, 3 min), peracetic acid (PAA, 0.5%, 3 min) and freezing at -25°C for 3 days up to 3 weeks. Intact bacteria were quantified by qPCR directly after treatment or after storage of the chicken skin for up to 3 weeks at 4°C. In parallel, CFU were determined on selective agar.

## 4. Results

The *E. coli uidA*-based viability qPCR was designed and validated on 146 *E. coli*/*Shigella* strains, including 22  $\beta$ -glucuronidase negative isolates and on exclusivity strains such as *E. albertii*, *E. hermannii* and *E. fergusonii*. The qPCR was proven specific and viable/dead differentiation was shown to be detectable at an at least 3 log<sub>10</sub> ratio of artificially killed versus viable *E. coli* using the internal sample process control developed for the thermophilic *Campylobacter* qPCR.

Surprisingly, the decontamination strategies comparably affected both species. PAA caused an immediate consistent loss of CFU and intact cells. LA, in contrast, led to an immediate concentration-dependent loss of CFU, but only to a minor reduction in the number of intact cells. Over a period of 3 weeks, LA-treated cells lost cell membrane integrity more rapidly than untreated cells. In addition, reduction of CFU by freezing appeared to overestimate the loss of viability, quantified by the viability qPCRs for both pathogens.

## 5. Conclusion

The comparative data for CFU and IPIU will enhance safety evaluations and the choice of most effective reduction strategies against fecal pathogens along the food chain.

---

## 245-FMV

Effect of calcium on specific genes of the proteolytic system of *Lactococcus lactis* starter strains

\*B. Forler<sup>1</sup>, A. Weiß<sup>1</sup>, H. Schmidt<sup>1</sup>

<sup>1</sup>University of Hohenheim, Department of Food Microbiology and Hygiene, Stuttgart, Germany

**Introduction:** The proteolytic system of dairy *Lactococcus lactis* strains enables them to cleave the milk protein casein into oligopeptides, which are subsequently transported into the cytoplasm and metabolized. The most important components of the proteolytic system are the cell envelope proteinase PrtP, the oligopeptide permease system Opp, and the transcriptional regulator CodY. The calcium contained in milk is hypothesized to affect the regulation of the *prtP* gene and thus to contribute to an increased release of peptides, some of which confer a bitter taste to calcium enriched fermented products.

**Objectives:** The aim of this work is to analyze the transcriptional patterns of the genes *prtP*, *oppA* and *codY* of *Lactococcus lactis* starter culture isolates during the metabolism of caseins by quantitative real-time PCR.

**Materials and Methods:** A *L. lactis* ssp. *cremoris* and a *L. lactis* ssp. *lactis* strain were cultured in a milk-citrate broth with and without 50 mM CaCl<sub>2</sub>. Samples were taken after 5 h and 7 h and total RNA was isolated. The RNA was then transcribed into cDNA and the gene copy numbers were determined with external plasmid standards in a quantitative real-time PCR assay.

**Results:** The activity of the genes *prtP*, *oppA* and *codY* was highest after 5 h and then decreased. The addition of calcium significantly reduced the transcription of all three genes after 5 h and 7 h. The strongest effect was observed after 7 h, whereby *oppA* was the most strongly downregulated and *codY* the weakest downregulated gene.

**Conclusion:** The results of the study showed that the transcription of *prtP*, *oppA* and *codY* were significantly

reduced under high calcium conditions. It needs to be investigated how this contributes to the increased bitterness of fermented dairy products.

## 246-FMV

### Typing of *Salmonella enterica* by Fourier Transform Infrared (FTIR) Spectroscopy

\*M. Cordovana<sup>1</sup>, N. Mauder<sup>2</sup>, S. Pongolini<sup>3</sup>, L. Soliani<sup>3</sup>, S. Ambretti<sup>4</sup>, M. Kostrzewa<sup>5</sup>

<sup>1</sup>University Hospital Policlinico Sant'Orsola-Malpighi of Bologna, Microbiology, Bologna, Italy

<sup>2</sup>Bruker Daltonik, Bremen, Germany

<sup>3</sup>Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Risk Analysis and Genomic Epidemiology Unit, Parma, Italy

<sup>4</sup>University Hospital of Bologna Policlinico Sant'Orsola-Malpighi, Microbiology, Bologna, Italy

<sup>5</sup>Bruker Daltonik GmbH, Bremen, Germany

#### Background

*Salmonella enterica* is one of the leading cause of foodborne diseases worldwide, and represents a major public health burden. Differentiation of *S. enterica* at intra-species level is crucial, for the epidemiological investigation and to control foodborne outbreaks, but also from the clinical point of view (typhoid/non typhoid serovars). Several methods are widely used for this purpose (serotyping, phage typing, molecular methods), but they all are laborious and cost-intensive. In this study, we investigated the discriminatory power of the Fourier transform Infrared spectroscopy for *S. enterica* typing, in comparison with classical serotyping methods based on somatic and flagellar antigens.

#### Methods

A total of 128 well characterized clinical, foodborne, veterinary and environmental *S. enterica* isolates (belonging to 19 different serogroups), were analysed by the IR Biotyper system (IRBT - Bruker Daltonik). Infrared absorption spectra were acquired in transmission mode from a bacterial suspension in water/ethanol, placed in three technical replicates on a silicon sample plate, and let air dry.

Using the IRBT software, spectra relation within a wavenumber range from 1300 to 800/cm was analysed applying hierarchical cluster analysis (HCA) with Euclidean metric and single linkage.

#### Results

Congruence of IR Biotyper HCA and O-serogroups was evaluated with adjusted Wallace algorithm, indicating that IRBT can predict the O serogroup with 95% accuracy. The 19 different serogroups were clustered separately with a threshold set at 0.20. In addition, at a lower threshold, further intra-serogroup clustering was possible for some serotypes (among them particularly relevant was the separation of *S. Typhi* from the other O:9 serotypes).

#### Conclusion

IR Biotyper enabled an accurate discrimination of *Salmonella enterica* isolates at serogroup level, and in some case also at serotype level (*S. Typhi* from other group D serovars, monophasic variant of *S. Typhimurium* within group B). This technique proved to be a reliable, fast, high throughput and low-cost alternative typing methodology, suitable for

epidemiological investigations in routine microbiology laboratories.

## 247-FMV

### Plasma Treated Water: From lab to prototype for fresh food safety

\*U. Schnabel<sup>1,2</sup>, J. Stachowiak<sup>1</sup>, P. Bourke<sup>2</sup>, J. Ehlbeck<sup>1</sup>

<sup>1</sup>Leibniz Institute for Plasma Science and Technology, Plasma LifeScience, Greifswald, Germany

<sup>2</sup>Technological University Dublin, 2Environmental Sustainability & Health Institute (ESHI), Dublin, Ireland

Fresh-cut produce like lettuce may contain a very high microbial load, including human pathogens. Therefore, the need for antimicrobial agents at post-harvest stages to prevent microbial growth is evident.

Sanitation steps based on non-thermal plasma opens up innovative food processing possibilities by application at different points along the food chain; for production, modification, and preservation, as well as in packaging of plant- and animal-originated food.

Plasma differs from the gaseous state of matter by a certain amount of free charge carriers caused by ionization processes of the gas atoms and molecules due to the supply of energy.

This talk describes innovations resulting in a pilot scale fresh produce processing system based on plasma treated water. The primary focus was on antimicrobial efficacy in line with the importance of food safety to the fresh produce processing sector. The treatment of natural products with changing parameters (size, surface, water content) is challenging for the design and optimization of plasma processes. To overcome these challenges, a specific plasma process based on microwave plasma operated with compressed air was established to deliver plasma processed air (PPA) as the antimicrobial agent to process water. This served to functionalize the water (PTW) with antimicrobial properties.

To successfully scale up the application, an understanding of the antimicrobial properties, the chemical composition of PTW and the food quality characteristics of color and texture was developed. The optimized PTW production and decontamination process was implemented into pilot-plant scale lettuce-washing thus demonstrating the industrial scalability and applicability.

## 248-ZHYV

### Independent bioinformatics methods in bacterial genomics: Clustering *Francisella tularensis* isolates reveals old structures in a new light

\*T. Homeier-Bachmann<sup>1</sup>, \*A. Busch<sup>2,3</sup>, M. Abdel-Gil<sup>3</sup>, A. Hackbart<sup>3</sup>, H. Hotzel<sup>3</sup>, H. Tomaso<sup>3</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, IfE, Riems, Germany

<sup>2</sup>University Hospital, KAI, Jena, Germany

<sup>3</sup>Friedrich-Loeffler-Institute, IBIZ, Jena, Germany

#### Introduction

*Francisella tularensis* the causative agent of the zoonotic disease tularemia and is a highly virulent, Gram-negative bacterial pathogen. Human infection occurs through infected blood-feeding arthropods or by ingestion or inhalation. *Francisella tularensis* is listed as a category A bioterrorism agent because the infectious dose is very low and the

resulting febrile disease may be severe to fatal. The two subspecies *Francisella tularensis* subsp. *tularensis* and *Francisella tularensis* subsp. *holarctica* are the major causes of tularemia in humans. Only the less pathogenic *Francisella tularensis* subsp. *holarctica* is endemic in Europe. It appears to be a re-emerging in Germany and underestimated.

## Objectives

We wanted to develop a genotyping and subsequent clustering strategy for *Francisella tularensis* for diagnostic purposes. For that more than 150 whole-genome sequences of *Francisella tularensis* subsp. *holarctica* isolates isolated in the years 2008–2018 in Germany were generated.

## Materials & methods

We sequenced a comprehensive panel of German isolates and applied optimized quality and assembly strategies. We chose open source, reference independent methods to optimize the level of discrimination. Using a recently described clustering algorithm, we exploit a novel approach to the clustering of bacteria. APC is preferable to others for assigning clades and can be used for rapidly typing strains when they arise. We compared that to the more commonly used like HierBAPS.

## Results

By combining a reference-independent SNP analysis and ANI (average nucleotide identity) with affinity propagation clustering (APC), we developed a significantly improved methodology allowing resolving phylogenetic relationships, based on objective criteria.

## Conclusion

These bioinformatics tools can be used as a general ruler to determine phylogenetic relationships and clustering of bacteria, exemplarily done here with *Francisella tularensis*. This can be applied on whole genomes of other bacteria. This should allow the rapid risk assessment in the setting of epidemics and outbreaks.

---

## 249-ZHYV

### "CoxBase" – a unique interactive genotyping database for Q fever (*Coxiella burnetii*)

\*D. Frangoulidis<sup>1</sup>, M. C. Walter<sup>1</sup>, T. Dandekar<sup>2</sup>, A. Helbich<sup>1</sup>, K. Förstner<sup>3</sup>, M. Fasemore<sup>1,2</sup>

<sup>1</sup>Bundeswehr Institute of Microbiology, München, Germany

<sup>2</sup>University of Würzburg, Würzburg, Germany

<sup>3</sup>TH Köln – University of Applied Sciences, ZBMed, Cologne, Germany

The Q fever German Interdisciplinary program for research (Q-GAPS) has committed itself to investigate unsolved questions relating to the epidemiology, immunology, pathogenesis, surveillance and control of *Coxiella burnetii*, the pathogen of Q fever while striving towards attainment of the "One health" concept.

In our sub-project "Genomic and internet based analysis of *Coxiella burnetii*" we aim at a modern approach of active surveillance, research and joint data integration on the causative agent of Q fever, *C. burnetii*. Currently we are conducting the integration of strain typing data from *C. burnetii* into an interactive and publicly available online information platform named "CoxBase" which offers quick

retrieval and phylogenetic analysis. Data from newly sequenced strains as well as information about typing results, like tandem repeats suitable for MLVA analysis, Multi Spacer Typing (MST) and SNP-based characterization, will be provided. In-silico typing from newly generated whole genome sequences is another feature to support genomic surveillance. CoxBase is an online platform designed to be capable of presenting metadata with meaningful visual context usable on all existing internet-capable devices ("responsive design" including mobile phones). The platform will also provide evolutionary tree drawing functions to aid researches in tracing samples of similar origins as well as spatial links to geographic maps that pinpoint exact location of isolates. CoxBase is being designed to be a unique tool in the future for all molecular epidemiological and surveillance work and analysis in Q fever

---

## 250-ZHYV

### Vitamin C alleviates acute enterocolitis in *Campylobacter jejuni* infected mice

S. Mousavi<sup>1</sup>, E. Thunhorst<sup>2</sup>, S. Kittler<sup>2</sup>, S. Bereswill<sup>1</sup>, \*M. M. Heimesaat<sup>1</sup>

<sup>1</sup>Charité - University Medicine Berlin, Institute of Microbiology, Infectious Diseases and Immunology, Berlin, Germany

<sup>2</sup>University of Veterinary Medicine Hannover, Institute for Food Quality and Food Safety, Hannover, Germany

Human foodborne infections with the zoonotic pathogen *Campylobacter jejuni* are on the rise and constitute a significant socioeconomic burden worldwide. The health-beneficial, particularly anti-inflammatory effects of vitamin C (ascorbate) are well known. In our actual preclinical intervention study, we assessed potential anti-pathogenic and immunomodulatory effects of ascorbate in *C. jejuni*-infected secondary abiotic IL-10<sup>-/-</sup> mice developing acute campylobacteriosis similar to humans. Starting 4 days prior peroral *C. jejuni*-infection mice received synthetic ascorbate via the drinking water. At day 6 post-infection, ascorbate-treated mice harbored slightly lower colonic pathogen loads and suffered from less severe *C. jejuni*-induced enterocolitis as compared to placebo control animals. Ascorbate treatment did not only alleviate macroscopic sequelae of infection, but also dampened apoptotic and inflammatory immune cell responses in the intestines that were accompanied by less pronounced pro-inflammatory cytokine secretion, whereas cell regenerative measures were promoted. Remarkably, the anti-inflammatory effects of ascorbate pretreatment in *C. jejuni*-infected mice were not restricted to the intestinal tract, but could also be observed in extra-intestinal compartments including liver, kidneys and lungs. In conclusion, due to the potent anti-inflammatory effects observed in the clinical murine *C. jejuni*-infection model, ascorbate constitutes a promising novel option for prophylaxis and treatment of acute campylobacteriosis.

---

## 251-ZHYV

### Risk Factors for Anti-Hantavirus and Anti-Leptospira-Seropositivity amongst Forestry Workers in Lower Saxony, Germany

\*C. Klier<sup>1</sup>, C. Princk<sup>1</sup>, M. Mylius<sup>1</sup>, K. Meyer-Schlinkmann<sup>1</sup>, S. Schmitz<sup>1</sup>, M. Monazahian<sup>1</sup>, A. Baillet<sup>1</sup>, S. Rettenbacher-Riefler<sup>1</sup>, J. Dreesman<sup>1</sup>

<sup>1</sup>Niedersächsisches Landesgesundheitsamt, Hannover, Germany

## Introduction:

Hantavirus-infections and Leptospirosis are rodent-borne zoonotic diseases, causing mild to severe clinical manifestations. In Germany laboratory diagnosis of these

pathogens and Hantavirus-related haemorrhagic symptoms are subject to mandatory reporting.

To learn more about both the prevalence of these pathogens and the disease-associated risk factors, we conducted a study among an at-risk group of forestry workers (FW) in Lower-Saxony (LS).

#### Method:

Sera sampled in 2016 among FW in LS, Germany, were screened for both anti-Hantavirus (H) IgG and anti-Leptospira (L) IgG according to manufacturer recommendations. Data on demographics, potential risk-factors and applied preventive measures were collected by standardized questionnaire. Data were analysed by univariate and multivariate logistic regression analysis using Stata.

#### Result:

In total 883 FW (789 male, 94 female), age 18 to 67 years (ys) participated. 7.5% of sera were H-positive and 4.8% L-positive, respectively. H-seropositivity was highest in age group 35 – 49 ys. L-seropositivity increased with age reaching 7.5 % in age group 65+ ys. One H-seropositive FW (of n=66) recalled earlier clinical diagnosis of Hantavirus-infection; none of the L-positive FW (n=42) recalled diagnosis of Leptospirosis.

A subset of 601 FW (89.4% male, 10.6 % female, median age 53ys) answered the questionnaire. Seropositivity was higher in male than female FW ( $p=0.04$ ).

Preliminary analysis revealed that working in the office ( $p=0.013$ ) and taking precautions when handling rodents ( $p=0.02$ ) are protective against H-seropositivity, whereas handling wood ( $p=0.09$ ) is associated with H-seropositivity. So far no specific risk factor associated with L-seropositivity could be identified in this at-risk group of FW. Further results will be presented.

#### Conclusion:

H- and L-Seroprevalences are within the average of other studies among FW. Findings indicate that there is missing awareness of both Hantavirus-infections and Leptospirosis, leading to underreporting. Results will be used to develop and implement measures to increase awareness towards these pathogens and concomitant infections amongst practitioners, at-risk groups and the public.

#### 252-ZHYV

##### IS26-mediated transfer of *bla*<sub>NDM-1</sub> as the main route of resistance transmission during a polyclonal, multispecies outbreak in a German hospital

\*R. Weber<sup>1</sup>, M. Pietsch<sup>1</sup>, A. Frühauf<sup>1</sup>, Y. Pfeifer<sup>1</sup>, M. Martin<sup>2</sup>, D. Luft<sup>2</sup>, S. G. Gatermann<sup>3</sup>, N. Pfennigwerth<sup>3</sup>, M. Kaase<sup>3,4</sup>, G. Werner<sup>1</sup>, S. Fuchs<sup>1</sup>

<sup>1</sup>Robert Koch Institute, Department of Infectious Diseases, Wernigerode, Germany

<sup>2</sup>SLK Kliniken Heilbronn GmbH, Institute for Infection Prevention and Clinical Hygiene, Heilbronn, Germany

<sup>3</sup>National Reference Center for Gram-negative pathogens, Department for Medical Microbiology, Göttingen, Germany

<sup>4</sup>University Medicine Göttingen, Institute for Hospital Hygiene and Infectious Diseases, Göttingen, Germany

#### Introduction

One of the most demanding challenges in infection control is the world-wide dissemination of multidrug-resistant bacteria in clinical settings. Especially the increasing prevalence of carbapenemase producing Gram-negative pathogens poses an urgent threat to public health, as these enzymes confer resistance to almost all  $\beta$ -lactam antibiotics including carbapenems.

#### Objectives

In this study, we report a prolonged nosocomial outbreak of various NDM-1-producing Enterobacterales species due to clonal spread and cross-species exchange of *bla*<sub>NDM-1</sub>-carrying plasmids and transposons.

#### Methods

Between July 2015 and September 2017, a total of 51 carbapenemase-positive strains were collected from 38 patients and three environmental sources in a German hospital. To investigate the clonal relationship and involved mechanisms of *bla*<sub>NDM-1</sub> gene transfer, conjugation assays, molecular typing and whole genome sequence analyses were performed.

#### Results

The metallo- $\beta$ -lactamase gene *bla*<sub>NDM-1</sub> was found to be present in 35 of 51 isolates of which seven additionally carried the carbapenemase gene *bla*<sub>KPC-2</sub>. KPC-2 could also be detected within 16 NDM-1 negative isolates. Core genome MLST revealed different clusters of closely related isolates of *E. coli*, *K. pneumoniae*, *C. freundii*, *M. morganii* or *E. cloacae* indicating clonal spread of NDM-1 producing bacteria. The detailed reconstruction of plasmids revealed that in all outbreak-associated isolates *bla*<sub>NDM-1</sub> was located on composite transposons similar to Tn125, that was previously described for *Acinetobacter baumannii*. In contrast to Tn125, these structures were flanked by IS26 elements, which could facilitate horizontal gene transfer. Moreover, identical plasmids were found to be shared by *E. coli* and *M. morganii* isolates.

#### Conclusion

Our results highlight the importance of detailed genome-based analyses for resolving complex nosocomial outbreaks that include both, clonal spread of MDR pathogens and inter-species resistance gene transfer. It remains to be elucidated if standard infection prevention precautions are as effective for preventing horizontal resistance spread as it is for controlling clonal dissemination of pathogens.

#### 253-ZHYV

Mutations of resistance-nodulation-cell division efflux pump regulators in tigecycline-resistant *Acinetobacter baumannii* observed in a worldwide surveillance study

\*K. Lucaßen<sup>1</sup>, C. Müller<sup>1,2</sup>, J. Wille<sup>1,2</sup>, K. Xanthopoulou<sup>1,2</sup>, M. Hackel<sup>3</sup>, H. Seifert<sup>1,2</sup>, P. G. Higgins<sup>1,4,2</sup>

<sup>1</sup>University of Cologne, Institute for Medical Microbiology, Immunology and Hygiene, Cologne, Germany

<sup>2</sup>German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Germany

<sup>3</sup>International Health Management Associates, Schaumburg, IL, United States

<sup>4</sup>German Center for Infection Research (DZIF), Partner Site Bonn-Cologne, Köln, Germany

**Question:** Previously, tigecycline resistance in *Acinetobacter baumannii* isolates from Southern Europe was found to be associated with mutations in resistance-nodulation-cell division (RND)-type efflux pump regulatory genes [1]. These mutations alter their regulatory function by inducing increased efflux pump expression. The objective of this study was to investigate the prevalence of alterations of these regulators among clinical isolates from a worldwide surveillance study.

**Methods:** 317 isolates were collected as part of the Tigecycline Evaluation Surveillance Trail 2012 and 2016 from 89 centres worldwide. TGC resistance was determined by broth microdilution using the EUCAST breakpoint for *Enterobacterales* (> 0.5 mg/L). Whole-genome sequencing was performed by Illumina MiSeq. RND-type efflux pump regulators *adeRS*, *adeN* and *adeL* were compared to TGC susceptible *A. baumannii* isolates. *In silico* detection of resistance genes and insertion sequences was performed using ResFinder and IS-Mapper.

**Results:** In this study 32 TGC-resistant isolates from North and Central America (n=8), South America (n=2), Asia (n=16), and Europe (n=6) were included, within which regulatory gene disruption by *ISAbi1* was found in *adeN* and *adeS*. In addition, premature stop codons in *adeN* caused by nucleotide insertions, deletions, or substitutions were detected. These mutations were found in European, American and in Asian isolates. Furthermore, for every investigated regulator amino acid substitutions were found. The previously identified substitutions D21V and D26N in *adeR* and D167N in *adeS* were also limited to Southern Europe (Greece, Spain) in the present study. No strain investigated in this study carried the TGC resistance determinant *tet(x)*.

**Conclusions:** In most TGC-resistant isolates disruption, truncation, or amino acid substitutions of RND-type efflux pump regulatory genes were found. The present surveillance study shows that these mutations can be found worldwide. Contrary to this, various amino acid substitutions were hitherto only identified in European isolates.

[1] Gerson *et al.* JAC 2018, 73: 1501-1508

## 254-ZHYV

Emergence of ceftazidime/avibactam resistance in KPC-3 producing *Klebsiella pneumoniae in vivo*

S. Göttig<sup>1</sup>, D. Frank<sup>1</sup>, E. Mungo<sup>1</sup>, A. Nolte<sup>1</sup>, M. Hogardt<sup>1</sup>, S. Besier<sup>1</sup>, \*T. A. Wichelhaus<sup>1</sup>

<sup>1</sup>University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt am Main, Germany

**Introduction:** *Klebsiella pneumoniae* carbapenemase (KPC) is the most prevalent class A serine  $\beta$ -lactamase conferring carbapenem resistance worldwide. Inhibition of  $\beta$ -lactamases is a well-established aid in order to restore the bactericidal activity of common  $\beta$ -lactam antibiotics against multidrug-resistant Gram-negative pathogens. Avibactam, the first diazabicyclooctane  $\beta$ -lactamase inhibitor in clinical use, is unique in the sense that in combination with ceftazidime it not only overcomes extended spectrum  $\beta$ -lactamase but also KPC mediated resistance.

**Objectives:** We present molecular and phenotypic characterization of ceftazidime/avibactam resistance in KPC-3 producing *K. pneumoniae* that emerged *in vivo* and *in vitro*.

**Materials & Methods:** Sequence analysis of *blaKPC-3* was performed from clinical and *in vitro* generated ceftazidime/avibactam-resistant *K. pneumoniae* isolates. Time-kill kinetics and the *Galleria mellonella* infection model were applied to evaluate the activity of ceftazidime/avibactam and imipenem alone and in combination.

**Results:** The ceftazidime/avibactam-resistant clinical *K. pneumoniae* isolate revealed the amino acid change D179Y in KPC-3. Sixteen novel mutational changes in KPC-3 among *in vitro* selected ceftazidime/avibactam-resistant isolates could be described. Time-kill kinetics showed the emergence of resistant subpopulation under selection pressure with either imipenem or ceftazidime/avibactam. However, combined selection pressure with imipenem plus ceftazidime/avibactam prevented resistance development and resulted in a bactericidal activity. Concordantly, the *Galleria mellonella* infection model revealed that monotherapy with ceftazidime/avibactam is prone to select for resistance *in vivo* and that combination therapy with imipenem displays a significant better survival.

**Conclusions:** Ceftazidime/avibactam is a valuable antibiotic against multidrug- and carbapenem-resistant Enterobacterales. Based on time kill kinetics as well as *in vivo* infection model we postulate a combination therapy of ceftazidime/avibactam and imipenem as a strategy to prevent ceftazidime/avibactam resistance development in KPC producing Enterobacterales *in vivo*.

---

## 255-ZHYV

Transmission Surveillance of Multi-drug Resistant (MDR-) *Escherichia coli* Using Fourier-Transform Infrared (FTIR) Spectroscopy and Whole Genome Sequencing as Strain Typing Methods

\*S. Vogt<sup>1,2</sup>, K. Löffler<sup>1,2</sup>, B. Bader<sup>1,2</sup>, S. Peter<sup>1,2</sup>, \*J. Liese<sup>1,2</sup>

<sup>1</sup>University Hospital Tübingen, Institute of Medical Microbiology and Hygiene, Tübingen, Germany

<sup>2</sup>German Center for Infection Research (DZIF), partner site Tübingen, Tübingen, Germany

**Background:** *Escherichia (E.) coli* is one of the most common pathogens involved in nosocomial infections. The spread of multi-drug resistant (MDR) strains in the community and within hospitals is a major concern because of limited treatment options. However, the amount of transmission of MDR *E. coli* occurring in the hospital is debated. Highly precise typing tools are therefore needed, which determine the relationship of two isolates to confirm or exclude a transmission event.

**Objective:** The aim of the study was to determine transmission events in our hospital. Also, Fourier transform infra-red (FTIR) spectroscopy was evaluated as a strain typing method in comparison to a whole genome sequencing (WGS) approach for typing *E. coli*.

**Material and Methods:** Possible transmission events were identified retrospectively over a one-year period by overlaying hospital room occupancy data and routine microbiology results. Only *E. coli* isolates that were MDR according to the MRGN classification were included. All isolates were analyzed by FTIR spectroscopy and whole genome sequencing (followed by multi-locus sequence type (MLST) extraction and the construction of a SNP based phylogeny) to determine their phenotypic and genetic relationship.



**Results:** 109 *E. coli* isolates were analyzed that belonged to 97 possible (sometimes bi-directional) transmission events involving 101 patients. Phylogenetic analysis revealed a very diverse strain distribution comprising 23 sequence types and 89 SNP-based clusters, which included 76 singletons. Only 7 (7.2 %) transmission events could be confirmed by WGS-based strain typing. FTIR spectroscopy had a low sensitivity for identifying related isolates but exhibited a high negative predictive value. Clustering of isolate spectra showed moderate concordance with the sequence type and SNP-based phylogeny.

## Conclusion:

Most putative transmissions of MDR *E. coli* in our hospital were not confirmed, when molecular methods were applied to compare the isolates. FTIR spectroscopy had limitations when determining the relationship of isolate pairs in this endemic and highly diverse setting.

---

## 256-EMV

### Bacterial N and P Cycle in Biological Soil Crusts of Temperate Forests of Germany

\*J. Kurth<sup>1,2</sup>, M. Albrecht<sup>3</sup>, U. Karsten<sup>3</sup>, S. Kublik<sup>2</sup>, M. Schlöter<sup>1,2</sup>, S. Schulz<sup>2</sup>

<sup>1</sup>Technical University Munich, Science Center Weihenstephan, Department of Ecology and Ecosystem Management, Chair of Soil Science, Freising, Germany

<sup>2</sup>Helmholtz Center Munich, Research Unit Comparative Microbiome Analyses, Munich, Germany

<sup>3</sup>University of Rostock, Applied Ecology & Phycology, Rostock, Germany

Soil phosphorus (P) pools are strongly driven by microbial activities and *vice versa* P pools shape bacterial communities and their functional potential. Biological soil crusts (biocrusts) represent a microbial hotspot for nutrient turnover. Microbes strive to maintain a stable intracellular nutrient budget. We hypothesize that the microbial potential of a higher P turnover in biocrusts depends on the initial P content as well as acquisition and mobilization of other nutrients like nitrogen (N). Consequently, we expect different abundance pattern of bacteria involved in P and N cycle in biocrusts compared to respective parent materials. This was tested by quantifying bacterial genes coding for proteins catalyzing P mineralization (*phoD*, *phnX*), solubilization (*gcd*) and uptake (*pstS* and *pitA*) as well as N mineralization (*chiA*, *apr*) and fixation (*nifH*) by using qPCR and relating the obtained data to stable and labile P and N pools. We compared biocrusts and bare soil of temperate beech (*Fagus sylvatica* L.) forests representing a gradient in soil texture, nutrient contents, and pH levels. As postulated, our data indicate an increase in relative abundance of bacteria involved in P turnover in biocrusts. This was accompanied by higher labile P contents in biocrusts, especially at sites where total P pools were low. However, the strategy to acquire P differed between the sites. For example, the bacteria harboring the *pstS* gene, which is induced under P starvation, were most abundant in biocrusts at the site with the lowest labile P content. In contrast, the site with lowest total P contents has a higher potential to utilize more stable phosphonates compared to phosphomonoesters as indicated by higher abundance of *phnX* compared to *phoD*. N mineralization was strongly correlated to P turnover when both labile N and P pools were increased. Interestingly, the abundance of *nifH* was highest in the bare soil where total P contents were highest. Even though the correlation of N and P turnover is strongest if their availability is comparable, the acquisition strategy strongly depends on soil properties.

## 257-EMV

### A bacterial isolate from the Black Sea oxidizes sulfide with manganese(IV) oxide

\*J. Henkel<sup>1</sup>, O. Dellwig<sup>1</sup>, F. Pollehne<sup>1</sup>, D. Herlemann<sup>1,2</sup>, T. Leipe<sup>1</sup>, H. Schulz-Vogt<sup>1</sup>

<sup>1</sup>Leibniz Institute for Baltic Sea Research Warnemünde (IOW), biological oceanography, Rostock, Germany

<sup>2</sup>Estonian University of Life Sciences, Centre for Limnology, Vehendi Village, Estonia

Bacterial oxidation of sulfide (H<sub>2</sub>S) with manganese oxide (MnO<sub>2</sub>) in suboxic waters of the Black Sea was investigated since the 1980s, where H<sub>2</sub>S oxidation could not be attributed to either O<sub>2</sub>, NO<sub>3</sub><sup>-</sup> or light [1,2]. Even though isolation attempts at that time have failed [3], it was still a highly suggested energy metabolism in redox transition environments of water columns and sediments [e.g. 4]. We were now able to isolate a bacterial strain from the Black Sea, which reduces particulate MnO<sub>2</sub> with H<sub>2</sub>S and other sulfur intermediates [5]. The isolate belongs to the genus *Sulfurimonas* and was named *Sulfurimonas marisnigri* as a reference to the origin of the species. With the cultured isolate we were able to proof the existence of this metabolism, which explains H<sub>2</sub>S oxidation together with autotrophic growth of *Epsilonbacteraeota* at the chemocline of the Black Sea [6]. The biological catalysation of this process may have significant impact on the formation and maintenance of suboxic waters, due to the faster oxidation of H<sub>2</sub>S in comparison to the abiotic reaction. Further, the activity of bacteria with the new metabolism may also foster the authigenesis of manganese carbonate, which precipitated during growth of *S. marisnigri* in culture experiments. The existence of this metabolism is a direct biological link between the manganese and sulfur cycles with impact on the biogeochemistry of stratified waters and sediments.

## References:

- [1] Murray *et al.* (1989), Nature.
- [2] Overmann *et al.* (1992), Limnology and Oceanography.
- [3] Jannasch *et al.* (1991), Deep Sea Research Part A. Oceanographic Research Papers.
- [4] Jost *et al.* (2010), FEMS Microbiology Ecology.
- [5] Henkel *et al.* (2019), Proceedings of the national academy of sciences of the United States of America.
- [6] Grote *et al.* (2008), Applied and Environmental Microbiology.

---

## 258-EMV

### Isolation and characterization of halo-tolerant iron-oxidizing bacteria, *Alicyclobacillus* spp

\*N. D. Huynh<sup>1</sup>, G. Haferburg<sup>1</sup>, S. Kaschabek<sup>1</sup>, M. Schlömann<sup>1</sup>

<sup>1</sup>Institute of Biosciences, Environmental Microbiology, Freiberg, Germany

Bioleaching applies acidophilic sulfur- and iron-oxidizing microorganisms to extract valuable metals from sulfidic low-grade ores or concentrates. This technology has become an economical alternative to conventional techniques of metal extraction. However, acidophilic microorganisms, in general, tend to be intolerant to high concentrations of chloride. Efforts on enrichments and isolations of salt-tolerant iron-oxidizing bacteria have been made due to the limitation of well-known acidophilic iron-oxidizers in bioleaching with the

presence of high chloride ions as well as the promising positive effect of chloride addition in bioleaching of metal sulfide. The isolate can oxidize ferrous iron in the presence of high NaCl concentration. Phylogenetic tree generated by the maximum likelihood method shows *Alicyclobacillus* spp is likely to be a novel halotolerant iron-oxidizing bacteria. In comparison to chemical leaching of chalcopyrite, bioleaching in the presence of *Alicyclobacillus* spp showed a better copper dissolution. This new isolate might be useful for bioleaching in the presence of high sodium chloride concentration or bioleaching with saline/ seawater.

---

## 259-EMV

Dissecting sedimentary microbial populations in reactive hot spots of the transition zone between groundwater and surface water of first-order streams

\*Z. Wang<sup>1,2</sup>, †J. Fernández<sup>3,4</sup>, M. Schwientek<sup>3</sup>, G. Barthel<sup>5</sup>, T. Lüders<sup>1</sup>

<sup>1</sup>University of Bayreuth, Bayreuth, Germany

<sup>2</sup>Technical University of Munich, Munich, Germany

<sup>3</sup>University of Tübingen, Tübingen, Germany

<sup>4</sup>Helmholtz-Zentrum für Umweltforschung GmbH – UFZ, Leipzig, Germany

<sup>5</sup>Helmholtz Zentrum München – German Research Centre for Environmental Health, Neuherberg, Germany

Inputs of nitrogen species and other agricultural contaminants into rivers heavily depend on direct influx via lower-order streams. Here, the transition zone between groundwater and surface water of lower-order streams often has a decisive impact on the removal of pollutants. In contrast to higher-order streams, lower-order streams do not have extensive hyporheic zones, and strictly localized redox activities between groundwater and surface water can be expected upon transition. However, little scientific attention has been placed on microbial activities and communities of such defined reactive "hot spots". In this study, streambed sediment samples from first-order streams adjacent to the agricultural field near Tübingen, Germany were investigated. Seasonal sampling was performed at different sediment depths along transects, where distinct functionalities and redox processes are expected. Here, we present first results on whether specific exfiltration zones act either as sinks or as secondary sources of stream nitrate loading. The diversity and the abundance of microbial communities from these reactive hot spots were investigated by qPCR of N-cycling genes and by PacBio sequencing of full-length 16S rRNA amplicons. Our results indicate that a strong coupling between microbial controlled N- and S-cycling processes, and that prominent controls of microbiology by geohydrology. Our newly acquired longitudinal and seasonal insights on the distribution and the activities of sediment microbiomes enable us better understand the role of the transition zone between groundwater and surface water on contaminant attenuation in water bodies largely neglected to date.

---

## 260-EMV

Targeted metagenomics of environmental bacteria

\*A. Grieb<sup>1</sup>, R. M. Bowers<sup>2</sup>, M. Oggerin<sup>1</sup>, D. Goudeau<sup>2</sup>, J. Lee<sup>2</sup>, R. R. Malmstrom<sup>2</sup>, T. Woyke<sup>2</sup>, B. M. Fuchs<sup>1</sup>

<sup>1</sup>Max Planck Institute for Marine Microbiology, Bremen, Germany

<sup>2</sup>DOE Joint Genome Institute, Walnut Creek, United States

Metagenomics and single cell genomics provide a window into the genetic repertoire of yet uncultivated microorganisms based on sequencing data. However, bulk metagenomics struggle with the assembly of genomes from complex samples and the link between metabolic functions and taxonomic identity is not always given. Single cell genomics on the other hand is largely based on random cell selection,

missing species of low abundance. We here developed a targeted mini-metagenomic pipeline that combines fluorescence *in situ* hybridization (FISH) with fluorescence activated cell sorting (FACS). Cells hybridized with a taxon specific FISH probe are sorted based on their fluorescence signal prior to whole genome sequencing. As the specificity of the FISH probe used for sorting constrains the taxonomic identity of the sorted cells, the obtained sequences are of reduced diversity which facilitates genome assembly. Based on genome annotation, we infer metabolic functions of the targeted group. In this study, we optimized the pipeline from FISH for flow cytometric cell sorting to genome sequencing. We chose hybridization chain reaction (HCR)-FISH as a FISH method that does not impair DNA quality and at the same time yields bright fluorescence signals. Using cultured strains of various taxonomic affiliations, we first optimized the HCR-FISH protocol and subsequently assessed the influence of different cell fixatives on the signal intensity and the quality of genome assembly. The optimized pipeline, running with ethanol fixed cells, was subsequently applied to a marine water sample with a genus-specific HCR-FISH probe. Sorted cells were successfully sequenced and assembled, resulting in mini-metagenome assembled genomes of a novel and yet undescribed flavobacterial candidate species, thriving in the North Sea.

---

## 261-EMV

A high-pressure concept for cultivation of not-yet-cultivated microorganisms from different subsurface ecosystems

\*T. Trabold<sup>1</sup>, \*M. Hügler<sup>2</sup>, R. Moeller<sup>3</sup>, A. J. Probst<sup>4</sup>, M. Könneke<sup>5</sup>, M. Huth<sup>6</sup>

<sup>1</sup>FAU Erlangen-Nürnberg, Chair of Energy Process Engineering, Nuremberg, Germany

<sup>2</sup>TZW: DVGW-Technologiezentrum Wasser, Microbiology and Molecular Biology, Karlsruhe, Germany

<sup>3</sup>German Aerospace Center (DLR e.V.), Cologne, Germany

<sup>4</sup>University of Duisburg-Essen, Department of Chemistry, Institute for Environmental Microbiology and Biotechnology, Essen, Germany

<sup>5</sup>University of Bremen, MARUM - Center for Marine Environmental Sciences, Bremen, Germany

<sup>6</sup>Humboldt University Berlin, Lebenswissenschaftliche Fakultät, Berlin, Germany

## Introduction

With a total number of approximately 1030, archaea and bacteria are the most prominent life forms on Earth. However, only about 10.000 of the estimated four million microbial species have been cultivated and are available as a pure culture today. The uncultured majority represents consequently a huge knowledge gap concerning their biochemical and biotechnological potential. The most crucial challenge is to offer the right parameters for the cultivation, concerning, e.g., carbon and energy sources as well as the physical, chemical and biological boundary conditions.

## Objectives

The BMBF-project *MultiKulti* addresses this huge field of uncultivable organisms focusing on the deep biosphere, including continental and marine ecosystems. These ecosystems share several features, like high pressures, low nutrient concentrations and low accessibility, leading to high sampling efforts and costs. The main objectives of this project are (1) Gathering of initial information and metadata of the investigated habitats (e.g. community composition, biogeochemical cycles, etc) in order to define parameters and conditions for subsequent investigations (2) Development of specialized cultivation and sampling devices, enabling to mimic environmental conditions in order to keep thriving environmental samples in the laboratories for

(3) execution of systematic and reproducible experiments in order to get pure cultures of so far uncultivated species.

## "Materials & methods"

The project synergises the know-how of biologists from different fields and engineers who are working on high pressure fermentation and in-situ probing.

## Results

The BMBF-project MultiKulti started in November 2019 at an experimental stage. The contribution will show details of the planned approach and describe the current status.

### 262-EMV

Life in the Freezer?! – A culture-independent approach to assess potential microbial colonization of liquid nitrogen storage tanks

\*F. Bajerski<sup>1</sup>, A. Bürger<sup>2</sup>, B. Glasmacher<sup>3</sup>, E. R. J. Keller<sup>4</sup>, K. Müller<sup>5</sup>, K. Mühldorfer<sup>5</sup>, M. Nagel<sup>4</sup>, H. Rüdell<sup>6</sup>, T. Müller<sup>7</sup>, J. Schenkel<sup>8</sup>, J. Overmann<sup>1,9</sup>

<sup>1</sup>Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Department Microbial Ecology and Diversity, Braunschweig, Germany

<sup>2</sup>Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Developmental Genetics, München, Germany

<sup>3</sup>Leibniz University Hannover, Institute for Multiphase Processes, Hannover, Germany

<sup>4</sup>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Department of Genebank, Seeland, OT Gatersleben, Germany

<sup>5</sup>Leibniz Institute for Zoo and Wildlife Research (Leibniz-IZW), Berlin, Germany

<sup>6</sup>Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany

<sup>7</sup>BioKryo GmbH, Sulzbach, Germany

<sup>8</sup>German Cancer Research Centre, Heidelberg, Germany

<sup>9</sup>Braunschweig University of Technology, Microbiology, Braunschweig, Germany

Biobanking and cryopreservation currently gain increasing importance in the life sciences, particularly for medical and biotechnological applications. While protocols for quality management have been established to guarantee safe and clean storage of biological samples, there are also anecdotal reports of bacterial isolates and sample contamination indicating that organisms may persist in liquid nitrogen (LN) storage tanks.

In order to assess the types of organisms occurring in LN storage tanks, we systematically screened bacteria, fungi, plant and human cells in different phases of LN storage tanks maintained in ten different biobank facilities. Using state-of-the-art microscopic and culture-independent molecular approaches (group-specific marker gene amplification and high-throughput sequencing of bacterial ribosomal genes), we aimed to infer possible routes of entry and to deduce suitable strategies for quality management.

Bacterial cell counts and 16S rRNA gene copy numbers in the LN phase were in the range of the negative controls and hence below the determined detection limit of  $10^2$  cells per ml and  $10^3$  gene copies per ml. In the ice phase formed underneath the lids or accumulating at the bottom, small numbers of bacteria of up to  $10^4$  cells per ml and up to  $10^6$  gene copies per ml, as well as Mycoplasma, or fungi occurred in detectable amounts. The microorganisms may enter the LN storage tanks via the technical environment (*Pseudomonas*, *Acinetobacter*, *Methylobacterium*) or the human microbiome (*Bacteroides*, *Streptococcus*,

*Staphylococcus*) or the stored material itself (*Elizabethkingia*, *Janthibacterium*) were identified as sources. Based on our results, the small bacterial load of ice and debris samples can be minimized by the reduction of ice formation and by using hermetically sealed sample containers.<sup>1</sup>

<sup>1</sup>Bajerski et al., 2019. Factors determining microbial colonization of liquid nitrogen storage tanks used for archiving biological samples, Applied Microbiology and Biotechnology, in print.

### 263-EMV

A breath of "fresh country air" from a microbiological point of view

\*I. Laufer<sup>1</sup>, H. Kühne<sup>2</sup>, S. Ratering<sup>3</sup>, U. Jäckel<sup>1</sup>

<sup>1</sup>Federal Institute for Occupational Safety and Health, Berlin, Germany

<sup>2</sup>Saxon state company for environment and agriculture, Radebeul, Germany

<sup>3</sup>Justus-Liebig-University, Institute of Applied Microbiology, Giessen, Germany

Not only climate specialists [1] are interested in microorganisms of ambient air. At composting plants or animal fattening industries airborne microorganisms are often discussed as causatives of adverse pulmonary health effects [2, 3] and microbial analyses of "background air" are needed as reference to assess workers' or local residents' bioaerosol exposures. However, until now, only a few molecular analyses of bacterial composition in outdoor air have been conducted. Therefore, this project aims to investigate daily background concentration and composition of 16S rRNA genes in rural outdoor air as well as their influential parameters. Standardized sampling methods were used to collect bioaerosols over one year at three different locations in Saxony, Germany. Bioaerosol samples were analysed with Digital Droplet PCR and metabarcoding.

Between Sept. 2018 and Sept. 2019 mean concentrations of  $5 \times 10^3$ ,  $9 \times 10^3$  and  $1 \times 10^4$  16S rRNA genes were present per m<sup>3</sup> of air (n=128-136). A deeper look revealed significant seasonal differences ( $p < 0.001$ ). In winter and spring mean concentrations were  $4 \times 10^3$  genes m<sup>-3</sup> and  $6 \times 10^3$  genes m<sup>-3</sup>, respectively, while mean concentrations in summer and autumn were both  $1 \times 10^4$  genes m<sup>-3</sup>. In contrast, the percentage of bacterial DNA out of total DNA in the air was highest in winter (12 %) and lowest in summer (5 %). First results of metabarcoding analyses showed that bacterial DNA seems to be dominated by genera such as *Romboutsia*, *Lactobacillus*, *Clostridium* and *Terrisporobacter*. This indicates that bacteria in the "country air" are mainly of animal origin. A significant negative correlation was found between concentration of 16S rRNA genes and humidity at all sampling sites and at all seasons. In general the results obtained by this monitoring campaign help to identify sources of airborne bacteria as well as factors affecting microorganisms in outdoor air and thereby to improve risk assessment of bioaerosol exposure.

[1] Huffman et al. (2013) Atmos Chem Phys, 13: 6151-6164.

[2] Martin E et al. (2013) Environ Sci: Processes Impacts, 15: 478-484.

[3] Wing S and Wolf S (2000) Environ Health Perspect, 108: 233-238.

MyD88-dependent expression of IRG1 is required for efficient control of *Coxiella burnetii* infection  
L. Kohl<sup>1</sup>, I. Hayek<sup>1</sup>, C. Daniel<sup>2</sup>, A. Bozec<sup>2</sup>, A. Lührmann<sup>1</sup>, \*R. Lang<sup>1</sup>  
<sup>1</sup>*Institute of Clinical Microbiology, Immunology and Hygiene, Erlangen, Germany*  
<sup>2</sup>*University Hospital Erlangen, Erlangen, Germany*

The zoonotic disease Q-fever is caused by the obligate intracellular, Gram-negative bacterium *Coxiella burnetii* that replicates within phagocytes. Q-fever often occurs as a self-limiting flu-like illness, but can also become chronic and develop into an endocarditis. A single nucleotide polymorphism in the TLR adapter molecule Myd88 is associated with the development of chronic Q-fever in patients. We employed mice deficient in Myd88 as an *in vivo* model for chronic Q-fever. The attenuated strain *C. burnetii* Nine Mile Phase II was injected intraperitoneally or intratracheally, followed by kinetic analysis of bacterial burden and cytokine gene expression in tissues. *Myd88*<sup>-/-</sup> mice had significantly higher *C. burnetii* load on days 5 and 20 after intraperitoneal infection. Additionally, after infection via the intratracheal, natural route, we observed increased bacterial dissemination from the lung to other organs in *Myd88*<sup>-/-</sup> mice. Moreover, *Myd88*<sup>-/-</sup> mice developed persistent infection of the lung up to day 120 after intratracheal infection, whereas most wild type mice cleared the infection by day 27. On the other hand, increased bacterial burden in *Myd88*<sup>-/-</sup> mice was accompanied by reduced granulomatous inflammation and lower expression of chemokines, of IFN $\gamma$  and of its target genes. Validation of several IFN $\gamma$ -induced candidate genes using knockout mice revealed an essential role for *Irg1*, encoding the enzyme converting citrate to itaconate, in restricting the replication of *C. burnetii* in macrophages *in vitro* and in lung tissue *in vivo*. Furthermore, *C. burnetii* infected *Irg1*<sup>-/-</sup> mice showed significant weight loss and increased pro-inflammatory gene expression in spleen and lungs. These findings indicate an important contribution of MyD88-dependent induction of *Irg1* in generation of a protective host response to *C. burnetii* and a role in the resolution of Q-fever.

## 265-IIZV

The role of the complement system and diabetes in invasive candidiasis

\*V. Harpf<sup>1,2</sup>, S. Kenno<sup>1,2</sup>, C. Speth<sup>1,2</sup>, G. Rambach<sup>1,2</sup>, V. Fleischer<sup>1</sup>, N. Parth<sup>1</sup>, C. Lass-Flörl<sup>1,2</sup>, R. Würzner<sup>1,2</sup>

<sup>1</sup>*Medical University Innsbruck, Institute for Hygiene and Medical Microbiology, Innsbruck, Austria*

<sup>2</sup>*Christian Doppler Laboratory for Invasive Fungal Infections, Innsbruck, Austria*

## Background

Complement is an important link between the innate and the adaptive immunity and has several crucial functions in first line defense against non-self structures. To avoid detrimental effects, complement activity is tightly controlled by several regulators, one of which is factor H (fH). *Candida albicans* (CA) has developed mechanisms to escape complement. One of these is to bind fH on its surface via the "high affinity glucose transporter 1" (Hgt1p), whose expression is glucose-dependent. Hence, we aimed to study the effect of glucose and complement on CA pathogenesis.

## Methods

Immunocompetent (wt), diabetic and C3-deficient (DC3) mice were intravenously infected with CA wild-type (wt) or a mutant lacking Hgt1p (Dhgt1). Survival and clinical status were monitored over 14 days. Immunological and

inflammation parameters and fungal load were analyzed at defined time points.

## Results

The murine model revealed that the CA wt strain is less virulent in immunocompetent mice than the Dhgt1 strain, with a lower risk of lethal outcome and a slower pathogenesis. No significant difference in virulence was detected between diabetic and wt mice infected with Dhgt1 or between diabetic mice infected with the different *Candida* strains. In contrast, CA wt showed a significant higher virulence in diabetic and, in particular, DC3 mice, compared to wt mice. This proves that diabetes is a relevant risk factor for a worsened outcome of candidiasis and the causal association to complement. The higher fungal load in the urine as a parameter of a pronounced kidney infection and consecutive inflammation confirmed the survival data. Furthermore, the higher granulocyte numbers correlated with the virulence of the different *Candida* strains.

## Conclusion

The complement system is linked to the virulence of CA in diabetic animals. This might be due to lower C3b deposition on the surface of CA. Despite this, the depletion of Hgt1p cannot be attributed to be beneficial for the survival in the murine model. An explanation might be that this depletion could lead to either an up-regulation of other fH-binding molecules or other virulence factors.

---

## 266-IIZV

The author has not agreed to a publication.

---

## 267-IIZV

**Surprisingly similar humoral immune response to *Cryptococcus neoformans* in patients with cryptococcal meningitis and in healthy people with presumed environmental exposure**

\*A. E. Greßler<sup>1</sup>, B. Schulze<sup>1</sup>, D. Volke<sup>2</sup>, U. Müller<sup>1</sup>, D. Piehler<sup>1</sup>, A. Grahnert<sup>1</sup>, C. Firacative<sup>1,3</sup>, P. Escandón<sup>4</sup>, R. Hoffmann<sup>2</sup>, G. Alber<sup>1</sup>

<sup>1</sup>*University of Leipzig, Institute of Immunology, Leipzig, Germany*

<sup>2</sup>*University of Leipzig, Center for Biotechnology and Biomedicine, Leipzig, Germany*

<sup>3</sup>*Universidad del Rosario, Translational Microbiology and Emerging Diseases, Bogotá, Colombia*

<sup>4</sup>*Instituto Nacional de Salud, Microbiology, Bogotá, Colombia*

*Cryptococcus neoformans*, the main agent causing systemic cryptococcosis in immunocompromised patients, is known to be ubiquitously present in the environment. We investigated the humoral immune response towards the fungus using sera from Colombian cryptococcal meningitis patients, with or without HIV infection, and healthy control persons. Surprisingly, sera of all groups showed similar titers of anti-cryptococcal IgG and IgM directed against a) the whole organism, b) fungal proteins, and c) capsular polysaccharides. Therefore, we conclude that the level of antigenic burden and the nature of microbial encounter (active infection vs environmental exposure) affects the anti-fungal humoral immune response only to a minor extent. This was further supported by a murine model of pulmonary cryptococcal infection, where susceptible wild type Balb/c mice developed comparable titers of anti-cryptococcal antibodies as resistant IL-4R $\alpha$ -deficient mice, despite greatly different fungal burden in the lung. Moreover, infection of wild type Balb/c mice with a wide range of different inoculants of *C. neoformans* resulted in only minor differences of anti-cryptococcal IgG titers. Therefore, we propose that exposure

of humans to low doses of *Cryptococcus neoformans* is sufficient to trigger a long-lasting humoral immune response towards the fungus. 2-dimensional immunoblotting was applied to further investigate the anti-cryptococcal protein response and reveal qualitative differences in the human humoral response between individual cryptococcosis patients and healthy persons. In total, 230 out of a total of 311 cryptococcal protein spots detectable in the 2D gel were recognized by the human sera investigated in our study (n=40), emphasizing the diverse repertoire of human anti-cryptococcal antibodies. Ongoing analysis is carried out on the nature of the immunoreactive antigens recognized by cryptococcosis patients (HIV+ and HIV-) and healthy control persons.

---

## 268-IIZV

The author has not agreed to a publication.

---

## 269-IIZV

Characterization of *Arcobacter* strains isolated from human stool samples – results from the prospective German prevalence study Arcopath

V. Brückner<sup>1</sup>, U. Fiebiger<sup>2</sup>, R. Ignatius<sup>3</sup>, J. Friesen<sup>3</sup>, M. Eisenblätter<sup>4</sup>, M. Höck<sup>5</sup>, T. Alter<sup>1</sup>, S. Bereswill<sup>2</sup>, \*M. M. Heimesaat<sup>2</sup>, G. Götz<sup>1</sup>

<sup>1</sup>Freie Universität Berlin, Institute of Food Hygiene, Berlin, Germany

<sup>2</sup>Charité - University Medicine Berlin, Institute of Microbiology, Infectious Diseases and Immunology, Berlin, Germany

<sup>3</sup>Labor 28, Berlin, Berlin, Germany

<sup>4</sup>Synlab MVZ, Berlin, Berlin, Germany

<sup>5</sup>Labor Limbach, Berlin, Berlin, Germany

**Background:** *Arcobacter* constitute emerging food- and waterborne pathogens causing gastroenteritis in humans, but the underlying mechanisms are only incompletely understood. We therefore characterized *Arcobacter* isolates derived from human stool samples that had been collected during a prospective prevalence study in Germany *in vitro*.

**Methods:** Thirty-six bacterial isolates belonging to the species *A. butzleri* (n = 24), *A. cryaerophilus* (n = 10) and *A. lanthieri* (n = 2) were genotyped by ERIC-PCR, the expression of 10 putative virulence genes was assessed and cytotoxic effects on the human intestinal cell line HT-29/B6 were analyzed applying the WST-assay.

**Results:** Genotyping revealed high genetic diversity within the species *A. butzleri*, *A. cryaerophilus* and *A. lanthieri*. Both, *A. butzleri* and *A. lanthieri* encoded for a large number of putative virulence genes, while less genes were detectable in *A. cryaerophilus* isolates. Notably, the three cytolethal distending toxin (CDT) genes *cdtA*, *cdtB* and *cdtC* were abundant in both *A. lanthieri* isolates. Furthermore, all *A. butzleri* and *A. lanthieri*, but only one of the *A. cryaerophilus* isolates exerted cytotoxic effects.

**Conclusions:** Our study provides evidence for the abundance of putative virulence genes in *Arcobacter* isolates and prominent cytotoxic effects of *A. butzleri* and *A. lanthieri* *in vitro*. The presence of *cdtA*, *cdtB*, *cdtC* in *A. lanthieri* points towards CDT secretion as potential mechanism underlying cytotoxicity as opposed to *A. butzleri*. However, The association of the *Arcobacter* virulence factors detected and human morbidity should be addressed in future studies.

---

## 270-IIZV

Efficacy of recombinant BCG vaccine candidates against *Mycobacterium bovis* infection in goats

\*H. Köhler<sup>1</sup>, N. Wedlich<sup>1</sup>, J. Figl<sup>1</sup>, E. Liebler-Tenorio<sup>1</sup>, K. von Pückler<sup>2</sup>, S. Mamerow<sup>1,3</sup>, M. Rissmann<sup>1</sup>, R. Ulrich<sup>3</sup>, C. Schröder<sup>3</sup>, L. Grode<sup>4</sup>, S. H. E. Kaufmann<sup>5</sup>, C. Menge<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institute of Molecular Pathogenesis, Jena, Germany

<sup>2</sup>Justus-Liebig-University Giessen, Small Animal Clinic – Radiology, Giessen, Germany

<sup>3</sup>Friedrich-Loeffler-Institut, Department of Experimental Animal Facilities and Biorisk Management, Greifswald, Germany

<sup>4</sup>Vakzine Projekt Management GmbH, Hannover, Germany

<sup>5</sup>Max Planck Institute for Infection Biology, Berlin, Germany

*M.bovis* and *M.caprae* cause tuberculosis (TB) in domestic and wild ruminants and are important zoonotic pathogens. BCG, an attenuated TB vaccine strain derived from *M.bovis*, exhibits limited efficacy in adult animals and man. To generate more potent live TB vaccines, recombinant BCG (rBCG) strain VPM1002 was genetically engineered to express listeriolysin (Hly) from *Listeria monocytogenes*, while simultaneously deleting urease C (rBCG  $\Delta$ ureC::hly+). Derivatives VPM1002  $\Delta$ pdx1 and VPM1002  $\Delta$ nuoG were further modified to improve safety and efficacy, respectively. Aim of the study was to investigate the efficacy of the recombinant BCG strains against intra-bronchial *M.bovis* infection in a goat model.

Four groups of goats (n=8) were vaccinated subcutaneously with either BCG, VPM1002 or its derivatives at 12 weeks of age; mock vaccinated goats served as control. Four months later, all goats were challenged intra-bronchially with 2x10<sup>3</sup> CFU of *M.bovis* and progress of infection was monitored until 5 months post infection. At necropsy, tissue samples were taken for bacterial culture, lungs fixed intra-thoracically and analyzed by Computed tomography (CT).

Subcutaneous injection of the vaccines induced local inflammatory responses which were least pronounced in the VPM1002  $\Delta$ pdx-1 group. After challenge, none of the goats was clinically ill and *M. bovis* was not shed from nose or by faeces. CT analysis unveiled lung lesions in all goats of the control, VPM1002, VPM1002  $\Delta$ pdx1 and VPM1002  $\Delta$ nuoG groups and in 6/8 BCG vaccinated animals. The extent of the lesions varied, with high numbers in control and VPM1002  $\Delta$ pdx1 vaccinated goats, reduced numbers in BCG vaccinated goats and lowest numbers in the VPM1002  $\Delta$ nuoG and VPM1002 group. *M.bovis* was re-isolated from the mediastinal and tracheobronchial lymph nodes. The proportion of culture positive animals in the groups corresponded with the average extent of lung lesions documented for the group.

In summary, genetic modification of BCG has generated derivatives which proved to be more effective (VPM1002 and VPM1002  $\Delta$ nuoG) or safer (VPM1002  $\Delta$ pdx1) when applied to protect goats from the detrimental effects of *M.bovis*-induced TB.

---

## 271-IIZV

The role of polymorphic membrane proteins (Pmps) in *Chlamydia psittaci* strains with different infectious potential

\*A. Favaroni<sup>1</sup>, A. Trinks<sup>1</sup>, C. Schnee<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institute of Molecular Pathogenesis, Jena, Germany

*Chlamydia psittaci* is the etiological agent for chlamydiosis in birds and can be transferred to humans by inhalation, causing severe systemic disease and pneumonia. *C. psittaci* infects a broad range of hosts and tissues; strains have been indeed isolated from both avian and mammalian hosts, and

they seem to have different infectious and zoonotic potential, with avian strains being more virulent.

Pmps are a family of chlamydial proteins. Several Pmps from different species are immunoreactive; moreover, Pmps from the human pathogens *C. trachomatis* and *C. pneumoniae* are adhesins. Pmps are highly heterogeneous, suggesting a correlation with tissue tropism and pathogenicity. *C. psittaci* harbors 21 Pmps, heterogeneously expressed during the infection.

Our hypothesis is that the different infectious potential and host tropism of avian and mammalian *C. psittaci* strains may be dependent on a different set of Pmp expression.

At first, the virulence of two avian and two mammalian strains was compared by testing the survival rate of embryonated eggs after infection.

Bioinformatic analyses of the Pmps of 10 avian and mammalian isolates confirmed a high degree of *pmp* gene variation, however, no correlation with host species was found. Thus, comparisons of Pmp expression profiles from different strains were conducted via RT-qPCR and showed that Pmps of the G group are expressed differently in the analyzed strains. Pmps 8G and 17G, selected as "variable" and Pmp22D, selected as "conserved", were recombinantly produced from one avian and one mammalian representative strain and their adhesion ability and relevance for the infection were tested.

Recombinant Pmp22D and Pmp8G showed strong adhesion to avian and mammalian cell lines and blocked a subsequent *C. psittaci* infection, suggesting their essential role in *C. psittaci*. Recombinant Pmp17G showed weaker binding and only to avian cells, with no relevant blocking ability towards infection of mammalian strains.

These data suggest that some Pmps might be essential for *C. psittaci* infection, while presence of other Pmps might lead to a different tropism of *C. psittaci* strains, possibly by acting on different receptor pathways.

## 272-ARV

Anaerobic enrichment cultures from the Guaymas Basin reveal an unexpected diversity of thermophilic alkane-oxidizing archaea

\*G. Wegener<sup>1</sup>, R. Laso-Pérez<sup>1</sup>, C. Hahn<sup>1</sup>, D. Benito-Merino<sup>1</sup>, H. Zehnle<sup>1</sup>, M. Liebeke<sup>1</sup>, A. Teske<sup>1</sup>, K. Knittel<sup>1</sup>

<sup>1</sup>Max Planck Institute for Marine Microbiology, Bremen, Germany

Saturated hydrocarbons are attractive energy and carbon sources for microorganisms, but the knowledge on anaerobic hydrocarbon oxidizers that represent the primary biological sink for these compounds in marine environments is sparse. Anaerobic methane activation proceeds via the formation of methyl-coenzyme M reductase to yield CoM-bound methyl-groups, which are oxidized to CO<sub>2</sub> via the reversed methanogenesis pathway. A CoM-type alkane activation was thought to be restricted to the C1-compound methane. Recently retrieved anaerobic thermophilic enrichment cultures that oxidize the C4-compound butane were however dominated by the archaeon *Ca. Syntrophoarchaeum* that form consortia with partner bacteria, similar as observed in the anaerobic oxidation of methane (Laso-Pérez et al., 2016, Nature). *Ca. Syntrophoarchaeum* contains highly divergent MCR types that allow activation of butane via alkyl-CoM formation. Metagenomic studies (Wang et al. 2019, Nature Microbiol.) and additional cultivation approaches (Chen et al.,

2019; Nature) indicate that MCR-based multi-carbon alkane metabolism is widely distributed within the Archaea. Here we present our latest findings on the diversity, substrate range and metabolic pathways of novel hydrocarbon-oxidizing archaea that we enriched from the hydrocarbon-rich hydrothermal sediments of Guaymas Basin (Gulf of California, Mexico). We cultured strains of the Syntrophoarchaeum clade that oxidize hexane, therewith extending the substrate range of this archaeal group towards larger compounds, and new thermophilic strains of the GoM-Arc1 clade that grow C2-compound ethane as substrate with before unexpected growth rates. Additionally we detected new groups of archaea that use MCR-like enzymes for the activation of hexadecane. Phylogenetic marker genes and the divergent Mcr type of those Archaea are found in various hydrocarbon-rich environments, suggesting a dominant role of archaea in anaerobic short-chain hydrocarbon oxidation also under moderate temperatures.

## 273-ARV

**New mode of methanogenesis observed in the coal degrading methanogen *Methermicoccus shengliensis***

\*J. Kurth<sup>1</sup>, M. Nobu<sup>2</sup>, H. Tamaki<sup>2</sup>, N. de Jonge<sup>3</sup>, J. L. Nielsen<sup>3</sup>, M. S. M. Jetten<sup>1</sup>, Y. Kamagata<sup>2</sup>, C. Welte<sup>1</sup>

<sup>1</sup>Radboud University, Microbiology Department, Nijmegen, Netherlands

<sup>2</sup>National Institute of Advanced Industrial Science and Technology, Bioproduction Research Institute, Tsukuba, Japan

<sup>3</sup>Aalborg University, Department of Chemistry and Bioscience, Aalborg, Denmark

The main source of the greenhouse gas methane are methanogenic archaea. This emphasizes the importance of those organisms for the global carbon cycle. Although methanogens have been studied for more than 111 years [1], a novel methanogenic pathway was recently discovered: the thermophilic methanogen *Methermicoccus (M.) shengliensis* is able to use a large variety of methoxylated aromatic compounds as substrates for methane generation [2-4]. Despite the significance and novelty of this unique archaeon a detailed analysis of its metabolism is still missing. Here, we used transcriptomic and proteomic methods to investigate the response to growth on methoxylated aromatics. As a result we observed that *M. shengliensis* uses a demethoxylation system that is more related to that from acetogenic bacteria than to the methyl transferase system used for methylotrophic methanogenesis. It activates methoxy-groups using tetrahydromethanopterin as the carrier, a mechanism distinct from conventional methanogenic methyl-transfer systems dependent on Coenzyme M. We further discovered that this leads to a two-phasic metabolism. In the first phase *M. shengliensis* disproportionates methoxy groups to CO<sub>2</sub> and CH<sub>4</sub> which leads to an excess of reducing equivalents. In the second phase CO<sub>2</sub> is reduced to CH<sub>4</sub> with help of the remaining reducing equivalents from phase one. This novel two-phasic mode of methanogenesis represents an unusual redox-cyclic metabolism.

[1] Soehngen NL (1906) [uuid:f736c111-841b-4afb-ac8f-21473d4d89ab](#).

[2] Mayumi D (2016) DOI:10.1126/science.aaf8821.

[3] Cheng L (2007) DOI: 10.1099/ij.s.0.65049-0.

[4] Welte C (2016) DOI:10.1126/science.aai8101.

---

## 274-ARV

Dispensable methanogenic energy conservation in

### *Methanosarcina acetivorans*

\*C. Schöne<sup>1</sup>, N. Jehmlich<sup>2</sup>, M. Rother<sup>1</sup>

<sup>1</sup>TU Dresden, Dresden, Germany

<sup>2</sup>Helmholtz-Zentrum für Umweltforschung, Molekulare Systembiologie, Leipzig, Germany

The biogenic formation of methane (methanogenesis) is a unique way of certain *Archaea*, to conserve energy, by coupling the pathway of methanogenesis to the generation of an ion motive force. To date, all known methanogenic archaea (methanogens) depend on methanogenesis for growth. *Methanosarcina acetivorans* can use carbon monoxide (CO) as an energy substrate and produces, beside methane, substantial amounts of acetate via a variant of the reductive acetyl-coenzyme A pathway. As this pathway involves ATP synthesis by substrate-level phosphorylation, the questions arose if *M. acetivorans* can grow on CO without the need to produce methane, and if the respiratory chain of the organism is still required. To address this question, the operon encoding the membraneintegral, energy-converting N<sup>5</sup>-methyl-tetrahydrosarcinapterin (H<sub>4</sub>SPT):coenzyme M (HS-CoM) methyltransferase (Mtr) was deleted. The resulting mutant still needed anabolic amounts of a methyl-group donor (e.g. methanol or trimethylamine) for growth on CO. Surprisingly, this requirement could be overcome by spontaneous mutation, resulting in a suppressor strain that generated essentially no methane from CO, and that lacked the energy-conserving membrane-bound heterodisulfide reductase (HdrED), which acts as the terminal reducing complex in the respiratory chain of cytochrome-containing methanogens. By deleting the HdrED-encoding genes in the suppressor strain it was confirmed that CO metabolism of *M. acetivorans* is independent of a substantial part of the respiratory chain. The various implications of these findings will be discussed.

---

## 275-ARV

Enriching and managing a hydrogenotrophic microbiome towards selective methane production during *ex situ* biomethanation

\*W. Logrono<sup>1</sup>, D. Popp<sup>1</sup>, H. Harms<sup>1</sup>, S. Kleinsteuber<sup>1</sup>, M. Nikolausz<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research – UFZ, Department of Environmental Microbiology, Leipzig, Germany

Biomethanation emerged as a plausible solution to the energy storage problem so that surplus electricity could be stored in a chemical fashion, that is, biomethane. By this, the anaerobic digestion technology (AD) could be integrated with other renewable energies. Biomethanation exploits the metabolic potential of hydrogenotrophic methanogens to convert H<sub>2</sub> and CO<sub>2</sub> into CH<sub>4</sub>. Here, we enriched a model hydrogenotrophic microbiome to investigate *ex situ* biomethanation, with methanogenesis and homoacetogenesis as competing reactions. Microbial community structure and dynamics of bacteria and archaea were evaluated through 16S rRNA and *mcrA* amplicon sequencing, respectively. We applied the concept of microbial resource management to steer the bioprocess towards selective production of CH<sub>4</sub>. Experiments were conducted under mesophilic conditions in closed bioreactors with H<sub>2</sub> (80%) and CO<sub>2</sub> (20%) as substrate. Acetate was concomitantly produced with CH<sub>4</sub> over several culture transfers. By controlling the media composition and the reducing agent the synthesis of this byproduct was reduced to ≤ 100 mg L<sup>-1</sup> and obtained grid quality biomethane (≥ 96%). Interestingly, we observed that formate was an intermediate during the bioprocess. The dominant

hydrogenotrophic methanogens belonged to the genera *Methanobacterium* and *Methanoculleus*. The bacterial community was dominated by the genus *Lutispora*. The only described species belonging to this genus is a thermophile not known to be a homoacetogen, which suggests that the AD black box microbiota needs to be further explored. We found that elevated stirring speed (≥ 1000 rpm) was detrimental, most likely due to the shear force. Collectively, the results suggest that model hydrogenotrophic microbiome enrichments are suitable for basic studies but also for efficient bioprocess. Microbial resource management for *ex situ* biomethanation is possible via controlling some medium components and operational parameters. In light of these findings, it seems possible to envision the development and control of microbiomes with desired functional traits via microbial resource management, yet this subject is in its infancy for biomethanation.

---

## 276-ARV

Microbial electrosynthesis of methane with different methanogens

\*F. Mayer<sup>1</sup>, F. Enzmann<sup>1</sup>, D. Holtmann<sup>1</sup>

<sup>1</sup>DECHEMA-Forschungsinstitut, Industrielle Biotechnologie, Frankfurt, Germany

### Introduction:

Microbial electrosynthesis (MES) provides a highly attractive perspective for the future generation of chemical products from electricity. Thereby electroactive microorganisms take up electrons from a cathode and convert CO<sub>2</sub> into chemical compounds.

### Objectives:

MES of methane with methanogens, also called electromethanogenesis, offers the chance to store electrical energy or excess current as chemical energy (biofuel). A possible application of MES might be the upgrading of biogas in its methane content. Therefore an electroactive biocatalyst is necessary.

### Materials & Methods:

So we screened several different methanogenic strains for its hydrogenotrophic growth and its ability to perform microbial electrosynthesis of methane. We analyzed the electron uptake at -700 mV (vs. standard hydrogen electrode) with chronoamperometric measurements and determined CO<sub>2</sub>, H<sub>2</sub> and CH<sub>4</sub> by gas analytics. Coulombic efficiency as well as the maximal current density was determined. The electrodes were examined with scanning electron microscopy for biofilm formation. Inhibition tests were performed to get insights into possible electron input modules during MES.

### Results:

We could show that the different strains *Methanococcus maripaludis*, *M. vanniellii*, *Methanobacterium congolense*, *Methanoculleus submarinus* and *Methanolacinia petrolearia* were able to take up electrons from a cathode and to produce methane via MES. Gas analysis revealed that first H<sub>2</sub> is produced from electrons and protons by the methanogens during MES. CO<sub>2</sub> is then converted to CH<sub>4</sub> with the biologically produced H<sub>2</sub>. Inhibition tests showed that hydrogenases are involved in electron uptake during electromethanogenesis. Obviously, *M. maripaludis* and *M. vanniellii* are the most resource efficient methane producer

during MES regarding the methane productivity ( $8.81 \pm 0.51$  mmol m<sup>-2</sup> d<sup>-1</sup>) and the Coulombic efficiency ( $58.9 \pm 0.8\%$ ).

## Conclusion:

Our results show that several methanogens are electroactive and can be used for the extracellular electron uptake from an electrode. With these methanogens it is possible to store electrical energy or excess current into the biofuel methane by MES.

## 277-ARV

Nanopore-based native RNA sequencing provides insights into prokaryotic transcription, operon structures, rRNA transcription, splicing, and RNA modifications

\*F. Grünberger<sup>1</sup>, R. Knüppel<sup>2</sup>, M. Jüttner<sup>2</sup>, M. Fenk<sup>1</sup>, A. Borst<sup>3</sup>, W. Hausner<sup>1</sup>, J. Soppa<sup>3</sup>, S. Ferreira-Cerca<sup>2</sup>, D. Grohmann<sup>1</sup>

<sup>1</sup>Institute of Microbiology, Department of Biochemistry, Genetics and Microbiology, Regensburg, Germany

<sup>2</sup>Biochemistry III, Department of Biochemistry, Genetics and Microbiology, Regensburg, Germany

<sup>3</sup>Goethe University, Institute for Molecular Biosciences, Frankfurt, Germany

In the last decade, next-generation sequencing (NGS) technologies revolutionized the field of microbiology. Using transcriptomics, important advances were possible in the field of RNA biology, which shaped our understanding of the transcriptional landscape in prokaryotes. However, for a long time, the analysis was limited by the short read lengths of Illumina-based sequencing and most likely biased by cDNA conversion.

Recently, Oxford Nanopore Technologies (ONT) developed a platform for highly parallel direct sequencing of native full-length RNAs. Here, we present the first native RNA-seq study of bacterial and archaeal transcriptomes using the Nanopore technology. We established an experimental workflow that includes the enzymatic polyadenylation of prokaryotic RNA to make them amenable for ONT's direct RNA sequencing kit. The accuracy and reliability of native RNA-seq in comparison to Illumina-based sequencing was evaluated by studying bacterial (*E. coli*) and archaeal transcriptomes (*Haloferax volcanii*, *Pyrococcus furiosus*). Based on this approach, multiple transcriptomic features could be addressed simultaneously: The analysis included determination of transcript boundaries. We moreover demonstrate that the long RNA reads gathered on the ONT platform allow reliable transcriptional unit assignment. Strikingly, we gained insights into the so far poorly understood rRNA splicing pathway in archaea and detected new splicing intermediates. Based on these data, we furthermore define hitherto unknown rRNA processing sites. As one of the key features of Nanopore native RNA sequencing is that RNA modifications are retained, we could confirm this ability by analyzing a well-known methylation site. Notably, by expanding the approach, we were able to show that rRNA modifications are more abundant in hyperthermophilic organisms.

Taken together, performing the first whole-genome native RNA-seq study in prokaryotes provided us with a wealth of information on transcriptional and post-transcriptional processes in prokaryotic model organisms. While some improvements certainly have to be made, we believe, that the technique is an important addition to the current NGS-toolbox.

## 278-ARV

### The DPANN way of life – insights into the interaction of a Micrarchaeon and a novel member of the Thermoplasmatales

\*S. Gfrerer<sup>1,2</sup>, S. Krause<sup>1</sup>, L. Villanueva<sup>3</sup>, B. Bunk<sup>4</sup>, U. Kuhlicke<sup>5</sup>, T. Neu<sup>5</sup>, C. Reuse<sup>6</sup>, K. Schmidt-Hohagen<sup>6</sup>, K. Hiller<sup>6</sup>, J. Gescher<sup>1,2</sup>

<sup>1</sup>Karlsruher Institut für Technologie, Institut für Angewandte Biowissenschaften - Abtl für Angewandte Biowissenschaften, Karlsruhe, Germany

<sup>2</sup>Karlsruher Institut für Technologie, Institut für Biologische Grenzflächen, Eggenstein-Leopoldshafen, Germany

<sup>3</sup>Royal Netherlands Institute for Sea Research, Microbiology & Biogeochemistry (MMB), Den Burg, Germany

<sup>4</sup>Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Bioinformatics, Braunschweig, Germany

<sup>5</sup>Helmholtz Centre for Environmental Research - UFZ, Magdeburg, Germany

<sup>6</sup>Technische Universität Braunschweig, Department of Bioinformatics & Biochemistry, Braunschweig, Germany

## Introduction

In 2013 a new superphylum within the archaea and the identifier DPANN (acronym of the first included phyla) was proposed. This superphylum includes archaea with extremely small cell and genome size, which result in limited metabolic capabilities. Therefore, symbiotic lifestyles are proposed for DPANN members. Although this superphylum is broadly distributed, most members were identified through culture-independent methods and consequently the knowledge about them is very limited. Of all the DPANN archaea, just representatives of the genus Nanoarchaeota can be cultivated to date.

Here we describe a stable co-culture, containing a representative of a second DPANN phylum, a member of the Micrarchaeota, and a novel member of the Thermoplasmatales.

## Objectives

The objective of this study was, to get insights into the interaction between the organisms in the co-culture. A combination of current methods was used to further characterize the way of life of the Micrarchaeon and shed light on the mysterious lifestyle of the DPANN superphylum.

## Results & Methods

Originally isolated from a biofilm, the growth in biofilm-like structures turned out to be mandatory for the Micrarchaeon-related organism. To further define its niche, lectin staining combined with CARD-FISH was used to identify sugar compounds of the matrix.

Genomic and transcriptomic analyses were used to identify metabolic and biosynthesis pathways. Furthermore, influences of the DPANN member on the Thermoplasmatales-related organism were examined on transcriptomic level. Metabolomic analysis revealed further insight into metabolic pathways of both archaea.

Finally, a lipid analysis was performed, which showed the same cell membrane composition in both organisms.

## Conclusion

The performed analyses unveiled the grade of dependency of the DPANN member within this community. This co-culture represents the only described stable cultivation of a member of the DPANN superphylum, apart from the Nanoarchaeota, to date and therefore gives the opportunity



to enlighten the microbial dark matter of this enigmatic organisms.

## 279-ARV

Protein glycosylation in the thermoacidophilic crenarchaeon *S. acidocaldarius* – resemblance and unique features to the eukaryal and bacterial protein glycosylation process

\*B. Meyer<sup>1,2,3</sup>, L. Kuschmierz<sup>1</sup>, S. Meckelmann<sup>4</sup>, J. Wingender<sup>5</sup>, H. Dorfmueller<sup>2</sup>, S. V. Albers<sup>3</sup>, B. Siebers<sup>1</sup>

<sup>1</sup>University Duisburg Essen, Biofilm Centre, Molekulare Enzymtechnologie und Biochemie, Essen, Germany

<sup>2</sup>University Dundee, Molecular Microbiology, School of Life Sciences, Dundee, United Kingdom

<sup>3</sup>Albert-Ludwigs-Universität Freiburg, Molecular Biology of Archaea, Freiburg, Germany

<sup>4</sup>University Duisburg-Essen, Faculty of Chemistry Applied Analytical Chemistry, Essen, Germany

<sup>5</sup>University Duisburg-Essen, Biofilm Centre, Aquatic Microbiology, Essen, Germany

Within the last decade, the archaeal phylogenetic tree has been dramatically expanded and reshaped, strengthening the hypothesis that the origin of Eukarya is rooted next to the archaeal TACK superphylum. Here we provide new insights into the N-glycosylation process of *Sulfolobus acidocaldarius*, a member of the Crenarchaeota within the TACK superphylum, and the closest eukaryal-related archaeon for which the N-glycosylation has been described.

The essentiality of the N-glycosylation have been demonstrated in *S. acidocaldarius*, however, the details of this biosynthesis process still need to be elucidated. Recently we identified that, in contrast to Euryarchaeota, the assembly of the N-glycan occur on dolichol di-phosphate (Dol-PP). In addition to this similarity with Eukarya, the N-glycan core of *S. acidocaldarius* resembles the eukaryal chitobiose N-glycan core (GlcNAc- $\beta$ 1-4-GlcNAc). Deletion studies and *in vitro* assays allowed the identification of the GTs AglH and Agl23 responsible for the first two biosynthesis steps of crenarchaeal N-glycosylation. Interestingly these two GTs resemble the eukaryal Alg7 and Alg13/14 enzymes synthesizing the N-glycan core, indicating a common evolutionary origin. To elucidate whether the assembly of the N-glycan requires, like in Eukarya, beside nucleotide-activated sugar donors also Dol-P activated sugars, the genome was analysed for the presence of known eukaryal Dol-P-glucose synthase (DPG) and Dol-P-mannose synthase (DPM) homologs. This search led to the identification of a DPM and a DPG candidate. While the deletion of the DPG homolog showed no effect on the N-glycosylation, the deletion of the DPM homolog resulted in the lack of the Dol-P-Man in the membrane as well as in a reduced N-glycan size. This demonstrates that indeed Dol-P activated sugars are involved in the archaeal N-glycosylation process. Analyses of the DPM mutant showed many phenotypical changes, including the loss of motility, enhanced cell-cell aggregation, reduced cell growth at elevated salt concentrations, and an altered biofilm formation. This results underline the importance of this post-translational modification for this thermophilic archaeon.

## 280-MPV

There are many routes to the Type IVB Secretion System (T4BSS)

\*S. Malmshheimer<sup>1</sup>, P. Kircheis<sup>1</sup>, I. Grin<sup>1</sup>, S. Wagner<sup>1</sup>

<sup>1</sup>Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), Tübingen, Germany

Using their T4BSS *Legionella pneumophila* translocates over 300 effector proteins into the host cell to promote intracellular colonization and survival. T4BSS effectors are targeted posttranslationally by their C-terminal signal sequence to the DotLMN coupling complex of the system. The targeting of some effectors is mediated by the T4BSS chaperones IcmSW and LvgA which seem to act as an adapter between substrates and DotLN. From there effector proteins are believed to be handed over to the ATPases of the system which introduce them into the secretion channel of the core complex [1].

Among T4- effectors soluble proteins as well as integral membrane proteins (TMD-effectors) can be found. The latter find their final destination in one of the host cell's membranes. For TMD-effectors the targeting pathway is unclear, since their hydrophobic segments can be recognized by the Signal Recognition Particle (SRP) during translation and promotes targeting to the bacterial inner membrane.

Bioinformatical analysis revealed that a subset of TMD-effectors possesses transmembrane segments of reduced hydrophobicity, whereby they can avoid SRP-targeting [2]. With the help of membrane fractionation followed by mass spectrometry of a *L. pneumophila* WT and  $\Delta$ T4SS mutant strain, we could show that other TMD-effectors of higher hydrophobicity are indeed targeted and inserted into the bacterial inner membrane.

Intriguingly, T4SSs have the ability to accept substrates from the cytoplasm, the inner membrane, and the periplasm [3]. Thus, we now aim to understand the mechanism of the different targeting pathways of effector subgroups to the T4BSS. To begin with, we would like to determine which proteins of the T4BSS are needed for a successful translocation of TMD-effectors into host cells by analyzing relevant T4BSS mutants in a translocation assay. Moreover, we would like to investigate which part of the effector protein (e.g. c-terminal signal sequence, chaperone binding region) is important in which step of the translocation event (e.g. targeting, substrate recognition).

[1] Ghosal et al., Chang, Y.-W., Jeong, K.C., Vogel, J.P., Jensen, G., 2018. bioRxiv 1–36.

[2] Krampen, L., Malmshheimer, S., Grin, I., Trunk, T., Luhrmann, A., de Gier, J.W., Wagner, S., 2018. Nat Commun.

[3] Galan, J.E., Waksman, G., 2018. Cell 172, 1306–1318.

## 281-MPV

The T4SS effector protein AnkG of *Coxiella burnetii*

modulates the 7SK snRNP complex to prevent apoptosis

\*A. Cordsmeier<sup>1</sup>, W. Schäfer<sup>1</sup>, M. Kraner<sup>2</sup>, U. Fischer<sup>3</sup>, A. Lührmann<sup>1</sup>

<sup>1</sup>Universitätsklinikum Erlangen, Mikrobiologie, Erlangen, Germany

<sup>2</sup>Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany

<sup>3</sup>Julius-Maximilians-Universität Würzburg, Würzburg, Germany

**Introduction:** The obligate intracellular bacterium *Coxiella burnetii* is the causing agent of the zoonotic disease Q fever. This disease is usually acquired by inhaling contaminated dust from animal feces, urine, milk-, or birth products. Q fever is mainly a self-resolving flu-like illness. However, Q fever might develop to an atypical pneumonia, hepatitis or endocarditis.

Inhibition of host cell apoptosis is crucial for *C. burnetii* to maintain its intracellular niche and, thus, bacterial survival. The anti-apoptotic activity of *C. burnetii* is mediated by a type IV secretion system (T4SS), which is required to inject effector proteins into the host cell. The T4SS effector protein AnkG is known to exhibit anti-apoptotic properties, which depend on its nuclear localization. How AnkG alters nuclear function is unknown.

**Objectives:** Unravelling the mode of action of AnkG within the nucleus.

**Methods:** We used different molecular biological methods including Co-immunoprecipitation, immunofluorescence, RNA-immunoprecipitation, RNA-Sequencing, and immunoblot analysis.

**Results:** Recently, we identified several host cell proteins involved in RNA metabolism as AnkG binding partners. Here, we focus on the binding of AnkG to one of these proteins, the nucleolar RNA helicase 2 (DDX21). Under cell death conditions DDX21 re-localizes into the nucleoplasm. Importantly, AnkG prevents DDX21 re-localization. DDX21 functions by modulating the composition of the 7SK snRNP complex and thereby influencing transcription activity. Our results demonstrate that AnkG also binds to the 7SK snRNP complex. Thus, we verify the interaction of AnkG with the scaffolding protein LARP7 and the 7SK RNA. Furthermore, we could demonstrate that AnkG leads to the release of the transcription factor p-TEFb from the 7SK snRNP complex and RNAseq experiments confirmed that AnkG alters transcription activity.

**Conclusions:** Our results suggest that the anti-apoptotic effector protein AnkG of *C. burnetii* inhibits host cell apoptosis by altering activity of the 7SK snRNP complex via releasing p-TEFb and, thus, influencing transcriptional activity.

---

## 282-MPV

### Through the wall. New insights into the mechanism of typhoid toxin secretion in *Salmonella* Typhi.

\*T. Geiger<sup>1,2</sup>, M. Lara-Tejero<sup>1</sup>, Y. Xiong<sup>3</sup>, J. E. Galán<sup>1</sup>

<sup>1</sup>Yale University, School of Medicine, Department of Microbial Pathogenesis, New Haven, CT, United States

<sup>2</sup>Max von Pettenkofer-Institut, LMU München, Medizinische Mikrobiologie und Krankenhaushygiene, München, Germany

<sup>3</sup>Yale University, School of Medicine, Department of Molecular Biophysics and Biochemistry, New Haven, CT, United States

**Introduction:** Protein secretion mechanisms are essential for the virulence of most bacterial pathogens. Typhoid toxin is an essential virulence factor for *Salmonella* Typhi, the cause of typhoid fever in humans. This toxin is unique in that it is only produced within mammalian cells, and it must be trafficked to the extracellular space before intoxicating target cells.

**Question:** An essential and poorly understood aspect of this transport pathway is the secretion of typhoid toxin from the bacterium into the *S. Typhi*-containing vacuole. Protein transport across the bacterial envelope is particularly challenging in Gram-negative bacteria, where to reach the outside, proteins must first move through at least three barriers, the inner membrane, the peptidoglycan layer, and the outer membrane

**Methods and Results:** We could show that typhoid toxin secretion requires its translocation to the trans side of the peptidoglycan layer at the bacterial poles for subsequent

release through the outer membrane. This translocation process strictly depends on TtsA activity, a specialized muramidase, which requires the localized editing of peptidoglycan at the bacterial poles by a specific LD-transpeptidase YcbB. This transpeptidase introduces specific L>D crosslinks between the stem peptides that join the glycan strands of the peptidoglycan layer. We could show that peptidoglycan substrate recognition by TtsA depends on a discrete domain within its carboxy terminus, which targets the enzyme to the bacterial poles to recognize YcbB-edited peptidoglycan. Comparison of the atomic structures of TtsA bound to its substrate and that of a close homolog with different specificity identified specific determinants involved in substrate recognition. Combined with structure-guided mutagenesis and *in vitro* and *in vivo* crosslinking experiments, this study provides an unprecedented view of the mechanisms by which a muramidase recognizes its peptidoglycan substrate to facilitate protein secretion.

**Conclusion:** These studies describe a novel protein secretion mechanism that is probably conserved in many other bacterial species

---

## 283-MPV

### SPI-1 translocated effectors prolong the intracellular survival of *Salmonella* Typhimurium in macrophages

A. L. Becker<sup>1,2</sup>, \*K. Ehrhardt<sup>1,2</sup>, M. Matern<sup>1</sup>, G. A. Grassl<sup>1,2</sup>

<sup>1</sup>Hannover Medical School, Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany

<sup>2</sup>German Center for Infection Research (DZIF), Partner Site Hannover, Hannover, Germany

*Salmonella* causes a variety of diseases ranging from self-limiting enterocolitis to severe systemic infections. Dependent on the serovar, 2-5% of immunocompetent individuals become chronic carriers thus representing a reservoir for transmission. The factors contributing to persistent *Salmonella* infection are incompletely understood. Despite the well-known role for the invasion of non-phagocytic cells, we found that the virulence factors encoded in the *Salmonella* pathogenicity island-1 (SPI-1 effectors) contribute to long-term intracellular survival of *Salmonella*. In bone marrow derived macrophages, *Salmonella* deficient in the translocation of all SPI-1 effectors ( $\Delta invA$  or  $\Delta sipC$ ) invades similarly to wild-type *Salmonella*, but it shows a diminished replication and an increased filamentous growth, a reaction of *Salmonella* to evade intracellular stress. TNF- $\alpha$ , CXCL1, and CXCL10 as well as nitrosative stress are increased by infection with *Salmonella*  $\Delta invA$  or  $\Delta sipC$  compared to wild-type *Salmonella*, whereas IL-1b and IL-6 are decreased. Cytotoxicity measured by lactate dehydrogenase release is decreased in *Salmonella*  $\Delta invA$  or  $\Delta sipC$  infection. Several deletion mutants for individual SPI-1 effectors were tested, but so far no single SPI-1 effector seems to promote the intracellular survival. This suggests that the survival is dependent on multiple effectors or effectors have redundant functions. In summary, besides invasion into non-phagocytic cells, SPI-1 effectors play a role in survival of *Salmonella* in macrophages.

---

## 284-MPV

### Visualization of *Yersinia enterocolitica* type III secretion during host-cell infection

\*M. Wolters<sup>1</sup>, M. Rudolph<sup>1</sup>, A. Carsten<sup>1</sup>, M. Aepfelbacher<sup>1</sup>

<sup>1</sup>University Medical Center Hamburg-Eppendorf (UKE), Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany

Type III secretion systems (T3SSs) are essential virulence factors of numerous bacterial pathogens. Upon host cell

contact the T3SS machinery assembles a translocation pore within host cell membranes that is required for entry of bacterial effector proteins. Visualization of T3SS structural components, the pore complex and effectors by fluorescence microscopy techniques is challenging for several reasons: i) Fusion proteins of T3SS substrates with fluorescent proteins like GFP, which are resistant to T3SS-mediated unfolding, are known to block the secretion process. ii) The small size of the structures of interest limits the applicability of conventional optical microscopy for resolving their biomolecular organization. iii) Intrabacterial proteins are not easily accessible for immunostaining with conventional IgG antibodies. We here demonstrate the application of self-labeling enzyme tags, split-GFP reporter system and small peptide tags in combination with recombinant single-domain antibodies (sdAbs) for imaging of T3SS components in fixed and live cell experiments. Self-labeling enzyme tags proved useful for tagging of structural T3SS components but were not suitable for effector imaging due to blockage of secretion. Split-GFP could be used for live-imaging of translocated effectors but was inefficient for imaging of pore proteins most likely due to a lack of sensitivity. Peptide tags in combination with sdAbs proved useful for imaging of structural T3SS components as well as the translocation pore without affecting protein function or secretion. In combination with super-resolution microscopy techniques like STED, STORM and MINFLUX these tools will likely enable the study of T3SS function with high spatial and temporal resolution.

## 285-MPV

Functional membrane microdomains coordinate staphylococcal heme acquisition

\*L. Adolf<sup>1</sup>, A. Jochim<sup>1</sup>, L. Kricks<sup>2</sup>, D. Lopez<sup>2</sup>, S. Heilbronner<sup>1</sup>

<sup>1</sup>University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, Tübingen, Germany

<sup>2</sup>Spanish National Research Council (CNB-CSIC), National Centre for Biotechnology, Madrid, Germany

Iron is an essential trace element and its levels are actively limited in humans, which is referred to as "nutritional immunity". To overcome this limitation, the pathogen *Staphylococcus aureus* imports iron-scavenging siderophores using the transport systems Hts, Sir, Fhu, and Sst. Additionally, the Lsd system allows acquisition of heme. All these transporters were shown to be located in "functional membrane microdomains" (FMMs), which are specific patches of the membrane containing special lipids. This suggests spatial proximity of the systems on the cell surface. In line with this, it is reported that the ATPase FhuA energizes Fhu, Hts, and Sir and we speculated that the same might be true for the Lsd-dependent heme acquisition.

Using the bacterial adenylate cyclase two-hybrid system, we showed that FhuA interacts directly with the heme-permease LsdF. Additionally, a  $\Delta fhuA$  deletion mutant was attenuated in the presence of heme/Hb as sole source of nutrient iron mimicking the deficits of a  $\Delta lsd$  deletion mutant. This confirmed the central importance of FhuA for all these systems. Furthermore, we found that the Lsd system is functionally dependent on the presence of FMMs. Both blockage of FMM formation by using a statin as well as mutation of the FMM-scaffolding protein FloA resulted in heme acquisition defects.

To examine the importance of FMMs for iron uptake further, we will use pull down analysis to investigate whether a direct interaction of the transporters with FloA facilitates incorporation into the FMMs.

Our experiments suggest that FMMs and appropriate membrane topology are crucial for iron acquisition and

propose statins as antibacterial agents blocking pathogen proliferation during disease.

## 286-MPV

**Identification of NWMN\_0364, a novel virulence regulatory protein relevant for SaeRS activation in *Staphylococcus aureus***

\*J. Bosselmann<sup>1,2</sup>, M. Kucklick<sup>1,2</sup>, S. Engelmann<sup>1,2</sup>

<sup>1</sup>TU-Braunschweig, Institute of Microbiology, Braunschweig, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Microbial Proteomics, Braunschweig, Germany

*Staphylococcus aureus* is a human commensal bacterium whose lifestyle can become pathogenic, especially in immunocompromised patients, which can lead to skin infections, endocarditis, etc. Nowadays the ability of *S. aureus* to form biofilms and the increasing incidence of antibiotic resistant variants cause serious problems not only in hospitals. Therefore, it is essential to understand the adaptation to and the interaction with the human host and its immune system. In this study, we identified an up to now hypothetical protein of *S. aureus* Newman, called NWMN\_0364, in a secretome analysis. 0364 is a lipoprotein and we asked whether this protein influences the bacterial virulence. Accordingly, we tested a deletion mutant of 0364 for its virulence ability by an infection assay, where human macrophages were infected with the wild type or mutant strain. Here, we observed that the mutant strain was only able to kill 50% of all macrophages while the wild type was able to kill about 95%. To find the reason for the reduced virulence, we examined the extracellular proteins of both strains with a GeLC-MS/MS proteomic approach. With this method, we could show that many virulence factors that are regulated by the two-component system SaeRS are reduced in the deletion mutant. Moreover, SaeP the negative regulator of SaeRS was also less abundant. To determine if this effect is due to genetic regulation of the SaeRS system we performed transcriptional analyses, which revealed significant reduced transcript levels of the *sae* operon as well as of SaeR regulated genes in the 0364 mutant. Interestingly, a co-immunoprecipitation experiment in vivo gave hints about an interaction between 0364 and SaeP. To confirm this interaction, we generated recombinant proteins of SaeP and 0364 and performed an interaction assay. Again, the results showed an interaction between 0364 and SaeP. All these results indicate that NWMN\_0364 is important for virulence regulation by activation of the two-component system SaeRS.

## 287-MPV

**The functional ClpXP protease of *Chlamydia trachomatis* requires distinct clpP genes from separate genetic loci**

\*S. Pan<sup>1</sup>, D. Thomy<sup>1</sup>, I. Malik<sup>1</sup>, B. Henrichfreise<sup>2</sup>, H. Brötz-Oesterhelt<sup>1</sup>, P. Sass<sup>1</sup>

<sup>1</sup>Interfaculty Institute for Microbiology and Infection Medicine, Microbial Bioactive Compounds, Tuebingen, Germany

<sup>2</sup>University of Bonn, Institute for Pharmaceutical Microbiology, Bonn, Germany

Bacterial Clp proteases are highly conserved and structurally versatile machines that play central roles in microbial physiology and virulence regulation of important human pathogens. Therefore, Clp proteases have attracted considerable attention as new antimicrobial and anti-virulence targets (1,2). For regulated proteolysis, associated Clp-ATPases recognize and unfold the dedicated protein substrates to feed them into the degradation chamber of the tetradecameric proteolytic core ClpP (3). While most

bacteria, including *Staphylococcus aureus* and *Escherichia coli*, encode a single *clpP* gene that is sufficient to build up the proteolytic core, some pathogens such as *Mycobacterium tuberculosis* and *Listeria monocytogenes* encode more than one ClpP homolog.

Here, we functionally characterized the ClpXP protease of *Chlamydia trachomatis*, an obligate intracellular human pathogen which carries two homologous *clpP* genes in different genetic loci. As a common feature among obligatory intracellular bacteria, *Chlamydiaceae* maintain a small genome size as a result of reductive evolution. Therefore, the existence of two *clpP* genes in *C. trachomatis* is striking and suggests their indispensability for survival. We show that ctClpXP is formed by a hetero-tetradecameric proteolytic core, composed of ctClpP1 and ctClpP2 that associates with ctClpX via ctClpP2 for regulated protein degradation. Furthermore, ADEP antibiotics interfere with ClpXP functions by both preventing the interaction of ctClpX with ctClpP1P2 and activating the otherwise dormant proteolytic core for unregulated proteolysis, validating ctClpXP as an antibacterial target (4).

Regarding genetic organization and enzyme function, chlamydial Clp protease is unique among the characterized Clp systems in bacteria to date as two distinct ClpP homologs from distant genetic loci have to team up to build a functionally active protease, suggesting a protease safeguard mechanism on the genomic level.

- 1) Raju *et al.* 2012. Nat Rev Drug Discov, 11:777–789
- 2) Frees *et al.* 2014. Int J Med Microbiol, 304:142–149
- 3) Kirstein *et al.* 2009. Nat Rev Microbiol, 7:589–599
- 4) Pan *et al.* 2019. Sci Rep, 9(1):14129

## 288-MEEV

### Specific drivers of soil bacterial activity

\*J. Sikorski<sup>1</sup>, Y. Shang<sup>1</sup>, M. Schrupf<sup>2</sup>, I. Schöning<sup>2</sup>, S. Marhan<sup>3</sup>, E. Kandeler<sup>3</sup>, R. S. Boeddinghaus<sup>3</sup>, K. Birkhofer<sup>4</sup>, N. Hölzel<sup>5</sup>, D. Prati<sup>6</sup>, M. Fischer<sup>6</sup>, V. H. Klaus<sup>7</sup>, T. Kleinebecker<sup>8</sup>, J. Overmann<sup>1</sup>

<sup>1</sup>Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Microbial Ecology and Diversity, Braunschweig, Germany

<sup>2</sup>Max-Planck-Institut für Biogeochemie, Jena, Germany

<sup>3</sup>Universität Hohenheim, Fachgebiet Bodenbiologie, Hohenheim, Germany

<sup>4</sup>Brandenburgische Technische Universität, Cottbus, Germany

<sup>5</sup>Universität Münster, AG Biodiversität und Ökosystemforschung, Münster, Germany

<sup>6</sup>University of Bern, Institute of Plant Sciences, Bern, Switzerland

<sup>7</sup>ETH Zürich, Zürich, Switzerland

<sup>8</sup>Universität Giessen, Giessen, Germany

Microbial functions in natural environments are driven by physiologically active cells. A large fraction of bacteria in soils has been found to be inactive, dormant, sporulated, or even dead, and hence may hardly contribute to ecosystem functions. This may bias the conclusions drawn from molecular studies which are based on extracted DNA. Methods to detect the active taxa and which can cost-effectively be applied to multiple samples on a high-throughput basis are scarce. Here we use 16S rRNA gene amplicon sequences generated from both extracted RNA and DNA. We developed a method which identifies those sequence variants (taxa) which show large rRNA:rDNA ratios which are not explained by the stochastic variations introduced by nucleic acid extraction, PCR, or sequencing reactions. By using suitable thresholds we determined the active bacterial taxa across 60 grassland soil plots from the German "Biodiversity Exploratories" ([www.biodiversity-exploratories.de](http://www.biodiversity-exploratories.de)). Generally, on each plot, the RNA based composition of the active community is more similar to the

DNA based community composition of the same plot but differs more strongly across plots. Our threshold revealed only 1-2% of all taxa to be active. Their taxonomic composition is not different from the overall community composition and they cover both rare and dominant taxa. Boosted linear modelling revealed pH, soil carbon and soil moisture to be the prominent drivers of community composition from both RNA and DNA extractions. In contrast to community composition, the proportion of active taxa is driven by completely different variables, namely C/N ratio, cumulative grass coverage, fine-root biomass but also earthworm counts. Also, the proportion of active taxa correlates positively with soil respiration. This suggests that variables such as pH, which affects community composition but apparently not the activity status of the cells, have less ecological relevance than previously thought. As our method enables us to identify variables which drive the activity status of specific but yet uncultured taxa, it also yields novel insights into the ecology of microbial dark matter.

## 289-MEEV

### Effect of the entry of antibiotic-resistant pathogens on the community composition and the spread of resistance

\*S. Heß<sup>1</sup>, K. David<sup>2</sup>, M. Virta<sup>3</sup>, T. Hiltunen<sup>3</sup>

<sup>1</sup>TU Dresden, Institute of Microbiology, Dresden, Germany

<sup>2</sup>TU Dresden, Dresden, Germany

<sup>3</sup>University, Helsinki, Finland

According to forecasts by health organizations, the number of deaths due to infections with antibiotic-resistant bacteria will rise from the current 9,000,000 to 10 million annually within the next 30 years (e.g. Review on Antimicrobial Resistance). In order to prevent this dramatic development, effective strategies are needed that take both human and veterinary medicine as well as the environment into account. Little is currently known about the transfer between these compartments. In particular, there is a lack of knowledge concerning the survival of the antibiotic-resistant cells and their role in the spread and maintenance of the resistance gene in the other compartment. Through the discharge of treated wastewater, antibiotic-resistant human pathogens are permanently introduced into aquatic ecosystems. To fill this gap of knowledge, microcosm studies lasting for 22 days were conducted to investigate their impact on the microbial community composition (assembled community consisting of 21 Gram-negative species). Additionally, the effect of subinhibitory antibiotic resistance genes on the uptake of the plasmid by the respective community members was tested. In the short term, the entry of the three different *Escherichia coli* strains harboring the naturally occurring RP4 plasmid has significantly altered the microbial community. The observed change and subsequent recovery was independent of which *Escherichia coli* strain was added. In contrast to community composition, however, the latter played an important role in the spread of the plasmid carrying the resistance genes. If a species was able to pick up the plasmid, it happened at the beginning of the experiment within the first three transfers (8 days). The probability of a plasmid being acquired by a species was directly dependent on its abundance. The presence of subinhibitory antibiotic concentrations had only a secondary effect on the speed of the plasmid spread. In conclusion, resistance determinants carrying cells transferred between compartments can play a crucial role as starting points for the spread of antibiotic resistance genes even if they are not able to survive in the new environment.

## 290-MEEV

A novel plant tumorigenic *Rhizobium* sp. associated with crown gall disease of rhododendron, as revealed through genomics

\*N. Kuzmanovic<sup>1</sup>, E. Idczak<sup>2</sup>, S. Wagner<sup>2</sup>, M. Götz<sup>2</sup>, C. Spröer<sup>3</sup>, B. Bunk<sup>3</sup>, J. Overmann<sup>3,4</sup>, K. Smalla<sup>5</sup>

<sup>1</sup>Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

<sup>2</sup>Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Plant Protection in Horticulture and Forests, Braunschweig, Germany

<sup>3</sup>Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

<sup>4</sup>Microbiology, Braunschweig University of Technology, Braunschweig, Germany

<sup>5</sup>Julius Kühn-Institut, Federal Research Centre for Cultivated Plants (JKI), Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany

Tumorigenic agrobacteria are causal agents of crown gall disease worldwide. Crown gall is an economically important plant disease occurring in various agricultural crops. Tumorigenic agrobacteria are commonly identified within the genera *Agrobacterium*, *Allorhizobium* and *Rhizobium*. These phytopathogens carry tumor-inducing (Ti) plasmids that are indispensable for the pathogenesis.

Although crown gall disease was reported to occur in rhododendron, little information on the causal agent is available. The primary objective of this study was to identify and characterize agrobacteria associated with the disease in Germany.

Draft whole-genome sequences of three representative tumorigenic strains isolated from crown gall tumors on rhododendron were obtained using the Illumina NextSeq 500 platform. One strain was also sequenced on the PacBio platform to obtain a high-quality complete chromosome and plasmid sequences.

Genome comparisons with reference *Rhizobiaceae* strains suggested that tumorigenic bacteria isolated from rhododendron tumors are most closely related to *Rhizobium tumorigenes*, a novel tumorigenic bacterium discovered recently on blackberry in Serbia. Analysis of genome relatedness and phylogenomic inference indicated that *R. tumorigenes* and novel rhododendron strains belong to separate species and form a homogenous and independent clade within the genus *Rhizobium*. Moreover, tumorigenic bacteria isolated from rhododendron carry a large Ti plasmid (~380 kb), that was different to those carried by *R. tumorigenes* and Ti plasmids described so far. The novel Ti plasmid has an atypical organization of T-DNA and virulence (*vir*) regions, and carries conjugal transfer genes unusual for Ti plasmids.

The results of this study highlight the diversity of tumorigenic agrobacteria and their Ti plasmids, and contribute to the better understanding of the etiology of the crown gall disease on rhododendron.

## 291-MEEV

Hells Bells – unique calcite formations that grow through microbial nitrate-driven sulfur oxidation

\*K. Leberecht<sup>1,2</sup>, S. Ritter<sup>3</sup>, M. Isenbeck-Schröter<sup>3</sup>, L. Klose<sup>3</sup>, W. Stinnesbeck<sup>3</sup>, J. Gescher<sup>1,2</sup>

<sup>1</sup>Karlsruhe Institute of Technology (KIT), Institute for Applied Biology (IAB), Department of Applied Biology, Karlsruhe, Germany

<sup>2</sup>Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces, Eggenstein-Leopoldshafen, Germany

<sup>3</sup>University of Heidelberg, Institute of Earth Sciences, Heidelberg, Germany

## Introduction

Extraordinary, subaqueous bell-shaped calcite formations were discovered in a cenote on the Yucatán Peninsula, Mexico. Due to their appearance in darkness in a water depth of 28-38 m and the vicinity of an anoxic, sulfidic halocline, these complex speleothems were named *Hells Bells* by the local diving community. Previous studies indicated that the Hells Bells calcified and grew underwater throughout the past thousands of years and potentially through microbially induced calcite precipitation.

## Objectives

To address the central question whether microbial metabolic activity is significantly involved in the growth of Hells Bells, we analyzed the microbial diversity throughout the complex water column of the cenote. Additionally, we focused on the identification of the microbial key players and their metabolic activity in the pelagic redoxcline, a 1–2 m thick zone of steep counter-gradients of oxidized and reduced chemical species where the speleothems still grow.

## Methods

The microbial diversity of the different sub-biotopes was determined via 16S rRNA-based amplicon sequencing. For the microbial community in the redoxcline, a detailed metagenomic and metatranscriptomic profiling was conducted.

## Results & Conclusion

Phylogenetic analysis of the microbiome of the cenote revealed that the redoxcline is a microbial transition zone between fresh water and the halocline, which is strongly dominated by Proteobacteria. Our metadata provides strong evidence that the microbial key players that thrive in the gradients of sulfide as electron donor and nitrate as electron acceptor belong to the order Hydrogenophiles as well as unassigned  $\beta$ -Proteobacteria. The metatranscriptome indicates that the oxidation of reduced sulfur species mainly halts after production of elementary sulfur particles instead of a complete oxidation to sulfate. Here, we hypothesize that the anaerobic microbial process of nitrate-driven sulfur oxidation promotes calcite precipitation in the hypoxic redoxcline by increasing the local pH through the consumption of sulfide, nitrate and carbon dioxide.

## 292-MEEV

Metatranscriptomic insights into syntrophic propionate metabolism in anaerobic digestion

\*S. Dykma<sup>1</sup>, C. Gallert<sup>1</sup>

<sup>1</sup>Hochschule Emden-Leer, Mikrobiologie/Biotechnologie, Emden, Germany

Short chain fatty acids are major intermediates in the mineralization of organic matter in methanogenic environments. The thermodynamically challenging degradation of propionate requires microbial cooperation between a bacterium and a syntrophic partner. *Candidatus Syntrophosphaera (Cloacimonetes)* together with *Smithella* and *Syntrophobacter* were regularly identified as the dominant syntrophic propionate-oxidizing bacteria (SPOB) in biogas reactors from wastewater treatment plants. Here, we targeted such system as a model to study propionate

metabolism of those three genera SPOB using metagenomics and metatranscriptomics. In laboratory-scale reactors fed with propionate as sole carbon source *Ca. Syntrophosphaera*, *Smithella* and *Syntrophobacter* were among the bacteria that showed highest transcriptional activity, however, with a distinct gene expression profile. The methylmalonyl-CoA pathway (MMC) for propionate oxidation was active in *Syntrophobacter* and a large fraction of MMC-transcripts were also assigned to *Ca. Syntrophosphaera*. Contrary to the MMC expressed in *Syntrophobacter*, essential genes coding for enzymes involved in energy conservation were not detected. Moreover, the *Ca. Syntrophosphaera* highly express amino acid fermentation genes that are unique among SPOB. Using the metatranscriptomic approach we could provide novel molecular insights into the C6-dismutation pathway for propionate oxidation that has been identified only in *Smithella* so far.

## 293-MEEV

### Complex food webs in groundwater coincide with high genetic potential for chemolithoautotrophy

\*M. Herrmann<sup>1,2</sup>, P. Geesink<sup>1</sup>, L. Yan<sup>1,2</sup>, R. Lehmann<sup>1,2</sup>, K. U. Totsche<sup>3</sup>, K. Küsel<sup>1,2</sup>

<sup>1</sup>Friedrich Schiller University Jena, Institute of Biodiversity, Aquatic Geomicrobiology, Jena, Germany

<sup>2</sup>German Center for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

<sup>3</sup>Friedrich Schiller University Jena, Institute of Geosciences, Jena, Germany

Absence of light-driven primary production, limited space and oxygen availability cause groundwater ecosystems to show lower food web complexity compared to other freshwater habitats. Chemolithoautotrophy in the subsurface provides additional input of carbon, however, its effects on groundwater food webs are poorly understood. Targeting SSU rRNA genes and genes involved in bacterial chemolithoautotrophy, we followed eukaryotic and bacterial communities in oligotrophic groundwater along a hillslope setting of alternating mixed carbonate-/siliciclastic bedrock in central Germany, covering both oxic and anoxic conditions. Across all sites, we found diverse protist communities including *Ciliophora*, *Cercozoa*, *Ichthyosporae*, *Centropheliozoa*, and amoeba. Correlation with hydrochemical parameters was less pronounced for eukaryotes compared to bacteria across the hydrogeologically compartmentalized flow system. 18S rRNA sequence data also pointed to the presence of metazoan top predators such as *Cyclopoida* (*Arthropoda*) and *Stenostomidae* (*Platyhelminthes*). Interestingly, molecular evidence of these organisms was only found in groundwater wells where abundances of functional genes associated with chemolithoautotrophy were 10 to 100 times higher compared to wells without these top predators. In wells closer to recharge areas with presumably increased inputs of soil-derived substances and biota, up to 85% of the eukaryotic sequence reads were affiliated with fungi, together with a low potential for chemolithoautotrophy. Our results suggest the existence of complex food webs with several trophic levels in oligotrophic groundwater. Chemolithoautotrophy appears to provide strong support to the proposed trophic interactions, as it could feed in additional biomass produced by light-independent CO<sub>2</sub>-fixation.

## 294-MEEV

### Predatory interactions between mycophagous amoeba and fungal pathogens

\*S. H. Lau<sup>1,2</sup>, S. Höfgen<sup>3</sup>, S. Radosa<sup>1,2</sup>, I. Ferling<sup>1,2</sup>, J. Sprague<sup>1,2</sup>, V. Valiente<sup>3</sup>, F. Hillmann<sup>1</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute (HKI), Junior Research Group Evolution of Microbial Interactions, Jena, Germany

<sup>2</sup>Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany

<sup>3</sup>Leibniz Institute for Natural Product Research and Infection Biology Hans Knöll Institute (HKI), Junior Research Group Biobricks of Natural Product Biosyntheses, Jena, Germany

Predatory interactions among microbes are considered to be a major evolutionary driving force for biodiversity, but may also have contributed to the acquisition and maintenance of microbial virulence determinants. The Amoebozoa are a major group of ubiquitous, eukaryotic micro-predators that commonly feed on microbial prey by phagocytosis and subsequent intracellular killing. However, their complex biology has often prevented species specific interaction studies at the laboratory scale. To study the impact of amoeba predation on fungi and elucidate recognition as well as killing mechanisms, we have recently isolated and characterized the amoeba *Protostelium aurantium*.

Food source screenings identified a wide food spectrum of ascomycete and basidiomycete yeasts, but also included filamentous fungi such as *Aspergillus fumigatus* which was killed *via* an active invasion to the fungal hyphae. Functional genomic data revealed that fungal copper and redox homeostasis were the primary targets during phagocytic interactions in *C. parapsilosis*. Gene deletions for the highly expressed fungal copper exporter *Crp1* and the peroxiredoxin *Prx1* confirmed their role in copper and redox homeostasis, respectively and further identified methionine biosynthesis as ROS sensitive target during predation. Fluorescence microscopy using GFP-labelled cells of *Candida parapsilosis* further revealed that the yeast prey was rapidly internalized and underwent lysis within minutes inside the phagolysosome of *P. aurantium*. To identify these lytic factors, we have adapted the culture conditions to grow the amoeba in shake flasks up to 1 L using *C. parapsilosis* as a single food source. Cellular extracts from such *P. aurantium* cultures reduced fungal viability in a concentration dependent manner due to rapid lysis of the yeast cells. Experiments to identify this lytic factor are currently ongoing and will not only elucidate the highly efficient killing strategies employed by this fungi specific predator, but may also provide a new perspective on antifungal therapy or biocontrol.

## 295-MEEV

### Horizontal transfer of bacteriocin micrococcin P1 genes among nasal *Staphylococcus aureus* strains

\*S. Krauss<sup>1</sup>, B. Krismer<sup>1</sup>, S. Heilbronner<sup>1</sup>, A. Peschel<sup>1</sup>

<sup>1</sup>Eberhard-Karls Universität Tübingen, Interfaculty Institute of Microbiology and Infection Medicine Tübingen - Infection Biology, Tübingen, Germany

The human nasal microbiota has recently emerged as a promising source for new antimicrobials. Steady competition for nutrients promotes the evolution of strategies to surpass competitors, for instance the production of antimicrobial active compounds.

Screening of a collection of nasal isolates for antimicrobial activity against epidemic *S. aureus* USA300 resulted in the identification of USA300-inhibiting *S. aureus* isolate D4-19. The strain also exhibited strong antimicrobial activity against other clinical relevant bacterial pathogens. Genes required for the antimicrobial effect were identified by transposon mutagenesis and are located on a 28 kb plasmid called pD4-19. The biosynthetic gene cluster shows low similarity to micrococcin P1 biosynthesis genes of *Macrococcus*

*caseolyticus* plasmid pBAC115. Mass spectrometry analysis confirmed that *S. aureus* isolate D4-19 but not the transposon mutant produces a compound with the mass of micrococcin P1.

This is the first report of micrococcin P1 production by *S. aureus* suggesting that nasal *Staphylococcus* strains can take up bacteriocin plasmids by horizontal gene transfer. Transformation of *S. aureus* RN4220 with pD4-19 enabled the production of micrococcin P1 in RN4220. Growth curves revealed that the newly acquired production of MP1 has a strong impact on RN4220 fitness. Daily passaging of *S. aureus* RN4220 pD4-19 generated an adapted mutant and genome sequencing will be performed to identify genomic alterations responsible for the observed fitness increase.

These results support the notion that the human nose is a rich source for new antimicrobials of high diversity and interchangeability, which evolve continuously by horizontal exchange and recombination. However, the impact of sudden antibiotic production on the cell metabolism and general fitness and resulting genetic adaptations need to be further investigated.

---

## 296-SeSiMV

### c-di-AMP metabolism and functions in *Streptomyces*

\*N. Tschowri<sup>1</sup>

<sup>1</sup>Humboldt-Universität zu Berlin, Mikrobiologie, Berlin, Germany

Balance of the nucleotide molecule cyclic di-AMP is crucial for bacterial adaptation to osmotic signals and virulence. Both, accumulation and reduction of c-di-AMP can have detrimental effects on microbial vitality. However, antibiotic-producing *Streptomyces* and many other Actinobacteria produce c-di-AMP but do not contain known proteins for c-di-AMP cleavage. Here, we show that DisA is the major diadenylate cyclase producing c-di-AMP in *Streptomyces venezuelae*. As a counterpart to DisA, we identified AtaC as the founding member of a novel class of highly conserved c-di-AMP-specific phosphodiesterases. AtaC homologs are widely distributed in Actinobacteria and are also present in pathogens such as *S. pneumoniae* and *M. tuberculosis*. Our biochemical and structural analysis revealed that AtaC is a monomer in solution, and that it binds Mn<sup>2+</sup> to specifically hydrolyze c-di-AMP to AMP via the intermediate 5"-pApA. Inactivation of *disA* renders *S. venezuelae* highly susceptible to ionic osmotic stress and accumulation of c-di-AMP in the *ataC* mutant strongly delays development. We show that c-di-AMP binds to the RCK-domain protein CpeA that interacts with the cation / proton antiporter CpeB. Altogether, characterization of AtaC and CpeA as novel components of c-di-AMP signal transduction pathways in Actinobacteria demonstrates that correctly balanced c-di-AMP is needed for vitality and coordinated differentiation in multicellular *Streptomyces*.

---

## 297-SeSiMV

### Regulation and Mechanisms - Metabolism of the Osmostress protectants Ectoine and Hydroxyectoine in *Ruegeria pomeroyi*

\*L. Hermann<sup>1</sup>, C. N. Mais<sup>2</sup>, A. Seubert<sup>2</sup>, G. Bange<sup>2</sup>, E. Bremer<sup>1</sup>

<sup>1</sup>Philipps University Marburg, Microbiology, Marburg, Germany

<sup>2</sup>Philipps University Marburg, Chemistry, Marburg, Germany

A wide range of microorganisms use ectoine and hydroxyectoine as osmotic stress protectants. Many *Bacteria* including the *Roseobacter* species can recycle these osmolytes by using them as carbon, nitrogen and energy

sources. Employing the marine species *Ruegeria pomeroyi* as a model organism, we set out to learn more about the mechanisms of the degradation of ectoines and the genetic regulation of these processes.

To do so we investigated the main regulator of the operon, EnuR, a GntR-type protein which binds to two specific operator sites upstream of the ectoine utilization gene cluster and acts as a transcriptional repressor as shown by reporter gene and bandshift assays. EnuR consists of a DNA-binding domain tethered to a large aminotransferase domain by a long flexible linker. The truncated aminotransferase domain could successfully be overproduced and still showed a strong yellow colour. This originates from a lysine residue covalently binding pyridoxal phosphate (vitamin B6, PLP) as a cofactor to detect the inducers and ectoine metabolites N- $\alpha$ -ADABA and diaminobutyric acid (DABA). By employing an EnuR mutant unable to bind PLP and the apo-form of EnuR, we could show that this regulator needs PLP to interact with the primary aminogroup of its inducers. To further assess the catabolic route of both ectoines, we heterologously produced the core catabolic enzymes EutDE of *R. pomeroyi* in *E. coli* and could show via HPLC analysis, that not only both enzymes are needed to degrade ectoines, but that actually both, ectoine as well as hydroxyectoine are degraded by EutDE. Ectoine degradation leads to the well known inducers N- $\alpha$ -ADABA and DABA, whereas hydroxyectoine degradation produces hydroxy-N- $\alpha$ -ADABA and hydroxy-DABA as further substantiated by NMR analysis.

Bioinformatic analysis suggests the widespread use and adaptations of this metabolic pathway, not only in the context of phylogenomic or habitat distribution, but also in the context of microbes capable of both, ectoine production and degradation.

### Recent Publications:

Schulz *et al.* (2017) Environ Microbiol 19: 926–946

Schulz *et al.* (2017) Environ Microbiol 19: 4599-4619

Czech *et al.* (2018) Genes 9: 177

---

## 298-SeSiMV

### Rapid adaptation of signaling networks in the fungal pathogen *Magnaporthe oryzae*

\*K. Bersching<sup>1</sup>, S. Bohnert<sup>1</sup>, C. Grünewald<sup>2</sup>, A. Yemelin<sup>1</sup>, K. Andresen<sup>2</sup>, S. Jacob<sup>1</sup>

<sup>1</sup>Institute of Biotechnology and Drug research gGmbH, Kaiserslautern, Germany

<sup>2</sup>Johannes Gutenberg-University Mainz (JGU), Institute of Molecular Physiology, Mainz, Germany

The high osmolarity glycerol (HOG) pathway for osmoregulation in the model organism *Magnaporthe oryzae* is an attractive signaling pathway to study the basics of fungal physiology. The HOG pathway consists of a phosphorelay system and a MAPK cascade enabling to adapt towards extracellular osmotic changes in the environment. The major osmolyte produced as an osmotic stress response is arabitol. Individual "loss of function" (lof)-mutants of the HOG pathway are impaired in osmoregulation. Lof-mutants of the HOG pathway are unable to produce arabitol and thereby being more sensitive towards osmotic changes.

Long-term cultivation at high osmolarity resulted in stable mutants that arose as individuals being restored in

osmoregulation from each of the individual lof-mutant. Within a relatively short time period, compared to millions of years of evolution, a rewiring of the signaling pathway in *M. oryzae* could be observed after 4 weeks. Interestingly, all of the "suppressor"-mutants found to be exclusively produce glycerol as a major compatible upon salt stress instead of arabinol. The "suppressors" are reestablished in osmoregulation and are able to memorize osmoregulation-ability even after growing weeks without stress. In addition, all "suppressor" mutants are again sensitive to Fludioxonil, suggesting that the HOG path or even its function has been rewired independently in each "suppressor" strain.

This phenomenon has been further investigated by DNA and RNA sequencing of  $\Delta$ *Mohog1*(suppressed) resulting in a set of candidate genes may be responsible for the rewiring of the osmoregulation pathway.

We aim to combine theoretical approaches to integrate sequencing data from genomics and transcriptomics with modern quantitative (phospho)-proteomic techniques. Furthermore, reversed molecular genetics will be used to validate the candidate genes or even other factors found to putatively promote or constrain rapid evolutionary adaptation. We are convinced that this phenomenon is important for the comprehension of rapid evolutionary processes in eukaryotes and can be applied to other organisms apart from *M. oryzae*.

### 299-SeSiMV

Spotlight on Fungal Rhodopsins: Green Light Sensors Meet Super-resolution Microscopy and Electrophysiology

\*S. Panzer<sup>1</sup>, S. Spath<sup>1,2</sup>, C. Stigloher<sup>2</sup>, U. Terpitz<sup>1</sup>

<sup>1</sup>Julius Maximilian University, Biotechnology and Biophysics, Würzburg, Germany

<sup>2</sup>Julius Maximilian University, Imaging Core Facility, Würzburg, Germany

Phytopathogenic fungi are often exposed to light, which strongly influences their physiology and behavior. Thus, they possess different photoreceptors, among them rhodopsins, which perceive green light that is prevalent in the phyllosphere. Fungal rhodopsins are of the microbial type and use all-*trans*-retinal as a chromophore. These proteins are widespread in the fungal kingdom, but up to date, very little is known about their biological role. In electrophysiological experiments, it was found that CarO from the ascomycete *Fusarium fujikuroi*, as well as UmOps1 (CarO-like rhodopsin) and UmOps2 (*Leptosphaeria maculans* rhodopsin (LR)-like) from the Basidiomycete *Ustilago maydis* act as proton pumps [1,2]. Strikingly, UmOps1 is influenced by acidic pH, which greatly enhances the pump activity. An additional supporting effect is visible in the presence of weak organic acids (WOAs) like acetate or the auxin indoleacetic acid (IAA), not only in UmOps1 but also in CarO [2,3] suggesting a potential role of CarO-like rhodopsins in the plant-fungus interaction. In contrast, UmOps2 is lacking both effects. We use patch-clamp measurements on functional mutants of CarO and UmOps1 to investigate the mechanisms underlying the WOA-induced increase of the pump activity. Moreover, we use state-of-the-art fluorescence microscopic techniques including 3D-SIM to localize the rhodopsins in fungal cells. CarO as well as UmOps1 were found in the plasma membrane whereas UmOps2 mainly localizes in the tonoplast. We further use correlative light and electron microscopy (CLEM) to visualize the rhodopsins in the subcellular context.

[1] García-Martínez *et al.*, Sci. Rep., 2015

[2] Panzer *et al.*, Front. Microbiol., 2019

[2] Adam *et al.*, Int. J. Mol. Sci, 2018

### 300-SeSiMV

**Identification and characterization of secondary active betaine/choline/carnitine transporters in *Acinetobacter baumannii***

\*J. Breisch<sup>1</sup>, B. Averhoff<sup>1</sup>

<sup>1</sup>Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt, Germany

**Introduction:** In the last decades the number of *Acinetobacter baumannii* infections of intensive care patients increased significantly, due to its multidrug resistance and its diverse persistence mechanisms. An extraordinary trait of *A. baumannii* is its high desiccation resistance which is based on perfect adaptation to dry or hyperosmotic conditions. One strategy to cope with osmotic stress is the uptake of compatible solutes such as betaine or its precursor choline from the environment via specific transporters. Interestingly, *A. baumannii* has a whole set of six transporters belonging to the betaine/choline/carnitine transporter family (BCCT).

**Objectives:** The aim of the study was the biochemical characterization of the BCCT transporters of *A. baumannii* and to address the role of these transporters in osmotic stress protection and adaptation to the human host.

**Materials & methods:** Heterologous expression of BCCT transporters, solute uptake and mutant studies.

**Results:** Uptake studies with radioactively labeled substrates revealed that three of the BCCT transporters are choline transporters whereas three mediate the uptake of betaine. Two of the choline specific and one betaine specific transporter are osmo-independent. One of the osmo-independent choline transporters is also responsible for carnitine uptake which is used by *A. baumannii* as sole carbon and energy source<sup>1</sup>. Furthermore, we identified a transcriptional regulator of the carnitine metabolic pathway.

**Conclusions:** In this study we identified and characterized six BCCT transporters of *A. baumannii*. Further, we showed that these six transporters have significant roles in: osmotic stress protection and carbon source assimilation. Taken together, the presence of multiple osmo-independent BCCT transporters and its function in metabolic adaptation linked with the high abundance of betaine, choline and carnitine in the human suggests a role of these transporters in persistence of *A. baumannii* in the human host.

1. Breisch J, Waclawska I, Averhoff B. 2018. Microbiologyopen. **8**: e752

### 301-SeSiMV

Phosphoglucosyltransferase is a key regulatory point in glycogen metabolism during nitrogen-chlorosis.

\*S. Doello<sup>1</sup>, K. Forchhammer<sup>1</sup>

<sup>1</sup>University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Tübingen, Germany

One the most frequent challenges cyanobacteria face in nature is nitrogen limitation. The non-nitrogen fixing cyanobacterium *Synechocystis* sp. PCC 6803 has developed a metabolic strategy to survive periods of nitrogen starvation by entering a state of dormancy, which is characterized by



degradation of the photosynthetic apparatus and most cellular components and the accumulation of reserve polymers. During the awakening from nitrogen starvation, *Synechocystis* passes through a heterotrophic phase before re-entering photoautotrophic growth in which they respire the polymers they have accumulated during the preceding starvation phase (1). Here, we investigated the switch from a maintenance metabolism to heterotrophy upon addition of nitrate. We found that glycogen is the substrate for respiration during this process. Interestingly, the key enzymes for glycogen catabolism are already expressed during nitrogen starvation, when glycogen is being synthesized, rendering cells ready for rapid resuscitation (2). These enzymes are present in their active form, but glycogen degradation only starts after the addition of nitrate. Initiation of glycogen degradation seems to depend on dephosphorylation (3), and activation, of the phosphoglucumutase. Overall, our results show the importance of the regulation of glycogen degradation for survival of nitrogen starvation.

(1) Klotz, A. *et al.* Awakening of a Dormant Cyanobacterium from Nitrogen Chlorosis Reveals a Genetically Determined Program. *Biol.* **2016**, 26, 2862–2872.

(2) Doello, S. *et al.* A specific glycogen mobilization strategy enables awakening of dormant cyanobacteria from chlorosis. *Plant Physiol.* **2018**, 177, 594–603.

(3) Spät, P.; Klotz, A.; Rexroth, S.; Maček, B.; Forchhammer, K. Chlorosis as a developmental program in cyanobacteria: the proteomic fundament for survival and awakening. *Mol. Cell. Proteomics.* **2018**, 17(9): 1650–1669.

### 302-SeSiMV

An Hfq-binding sRNA controlling fatty acid metabolism in *Vibrio cholerae*

\*M. Huber<sup>1</sup>, K. Fröhlich<sup>1</sup>, K. Papenfort<sup>1</sup>

<sup>1</sup>Friedrich Schiller University, Institute of Microbiology, Jena, Germany

Small RNAs (sRNAs) are a versatile group of regulators affecting bacterial gene expression at various levels. The largest class of sRNAs function by short and imperfect base-pairing with trans-encoded target mRNAs, modifying their translation and / or the stability. A vast majority of these sRNAs require the global RNA chaperone Hfq to fulfill their function. Loss of Hfq typically results in pleiotropic phenotypes and in *Vibrio cholerae*, a major human pathogen, Hfq inactivation has been linked to reduced virulence, failure to produce biofilms, and impaired intercellular communication. However, the set of Hfq-bound RNAs in *V. cholerae* is currently unknown. In this project, we use RIP-seq analysis (RNA immunoprecipitation followed by high throughput sequencing) to detect RNA-ligands of Hfq in *V. cholerae*. We discovered 682 transcripts interacting with Hfq, 85 of which are small regulatory RNAs. Interestingly, 46 of these sRNAs originate from the 3' untranslated region (3' UTR) of coding transcripts. Detailed investigation of one of these sRNAs, called FarS (fatty acid regulated small RNA), identified a feed-forward loop regulating the transition between fatty acid biosynthesis and degradation. FarS is expressed from the 3' UTR of the *fabB* gene, whose gene product catalyzes the initial step in fatty acid biosynthesis, and expression of FarS is strongly repressed in the presence of fatty acids. We show that FarS inhibits the translation of two paralogous *fadE* mRNAs, encoding acyl-CoA dehydrogenase, the enzyme responsible for the first step in fatty acid degradation. Based on our findings, we

hypothesize that FarS is a central regulator of fatty acid metabolism in *V. cholerae*.

### 303-SeSiMV

The critical role of Zur and SmtB in regulating zinc homeostasis of *Mycobacterium smegmatis*

\*E. Goethe<sup>1</sup>, K. Laarmann<sup>1</sup>, J. Lührs<sup>1</sup>, M. Jarek<sup>2</sup>, J. Meens<sup>1</sup>, R. Goethe<sup>1</sup>, A. Lewin<sup>3</sup>

<sup>1</sup>University of Veterinary Medicine Hannover, Institute for Microbiology, Department of Infectious Diseases, Hannover, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Genome Analytics, Braunschweig, Germany

<sup>3</sup>Robert Koch Institute, Berlin, Germany

#### Introduction

Maintenance of zinc homeostasis is crucial for bacterial viability. Bacteria efficiently regulate zinc homeostasis by using transport- or storage-systems, such as the common ZnuABC importer, the ZitA exporter, or alternative zinc independent ribosomal proteins (ARP). In mycobacteria, these systems are regulated by the antagonistically working repressors SmtB and Zur. The knowledge of zinc metabolism in mycobacteria including the apathogenic species *Mycobacterium smegmatis* (MSMEG) is poor. Therefore, in the presented study we intended to provide a comprehensive overview of MSMEG zinc homeostasis.

#### Materials & Methods

RNA deep sequencing and transcriptome analyses of MSMEGΔ*smtB*, MSMEGΔ*zur*, MSMEGΔ*smtB*Δ*zur* deletion mutants and MSMEG wildtype from standard, TPEN or zinc culture. Zur-Box analyses, promoter studies of the *smtB*-promoter and Zur/SmtB ChIP assay.

#### Results

Transcriptome analyses of TPEN treated MSMEGwt and MSMEGΔ*zur* revealed an exceptional genomic clustering of strongly induced genes, *msmeg\_6045-6071*, including genes of ARPs (*msmeg\_6065-6068* and *msmeg\_6070*), the common zinc importer ZnuABC (*msmeg\_6047-45*) and three additional putative zinc transporters (*msmeg\_6051-49*, *msmeg\_6052*, *mepD*). The number of genes affected by zinc excess and deletion of *smtB* was considerably lower. The most prominently regulated gene in MSMEGΔ*smtB* was *msmeg\_0755*, a homologue to MTB ZitA. Transcriptomics revealed interdependent regulation of the *smtB-zur* operon by Zur and SmtB, as deletion of one regulator resulted in an increased expression of the other. ChIP assay showed zinc dependent binding of SmtB and Zur to the MSMEG *smtB* promoter. Promoter studies with MSMEGwt and mutants confirmed sensitive zinc dependent co-regulation of the *smtB-zur* operon by both regulators. This is in striking contrast to pathogenic mycobacteria, where *smtB-zur* is regulated by SmtB only.

#### Conclusion

Our works show that MSMEG is well adapted to respond to environmental zinc changes and revealed significant differences to pathogenic mycobacteria. This reflects the adaptation of each species to their specific environment and should be taken into account, when performing research in MSMEG.

### 304-PMV

#### Syntrophy and coexistence in a sulfide-oxidizing phototrophic bloom – Uncultured *Chlorobi* drive microbial community dynamics and sulfur cycling in a hypoxic habitat

\*S. E. Ruff<sup>1</sup>, S. Bhatnagar<sup>2</sup>, E. S. Cowley<sup>3</sup>, S. H. Kopf<sup>4</sup>, S. Pérez Castro<sup>1</sup>, S. Kearney<sup>5</sup>, S. C. Dawson<sup>6</sup>, K. Hanselmann<sup>7</sup>

<sup>1</sup>Marine Biological Laboratory, Ecosystems Center, Woods Hole, United States

<sup>2</sup>University of Calgary, Biosciences, Calgary, Canada

<sup>3</sup>University of Wisconsin-Madison, School of Medicine and Public Health, Madison, United States

<sup>4</sup>University of Colorado, Geological Sciences, Boulder, United States

<sup>5</sup>Massachusetts Institute of Technology, Civil and Environmental Engineering, Cambridge, United States

<sup>6</sup>University of California Davis, Microbiology and Molecular Genetics, Davis, United States

<sup>7</sup>Eidgenössische Technische Hochschule, Earth Sciences, Zürich, Switzerland

Lagoons are common along coastlines worldwide and important for biogeochemical element cycling, coastal biodiversity, coastal erosion protection and blue carbon sequestration. Here, we investigated a shallow lagoon in New England. The sulfido-methanogenic ecosystem releases hydrogen sulfide particularly upon physical disturbance, causing blooms of anoxygenic sulfur-oxidizing phototrophs. To study the habitat, microbial community structure, assembly and function we performed in situ experiments investigating the bloom dynamics over time.

We describe coexistence patterns and ecological niches in planktonic blooms of phototrophs that are similar to those found in phototrophic microbial mats. The water column showed steep gradients of oxygen, pH, sulfate, sulfide, and salinity. The upper part of the bloom was dominated by *Cyanobacteria*, the middle and lower parts by anoxygenic purple sulfur bacteria (*Chromatiales*) and green sulfur bacteria (*Chlorobiales*), respectively. We show stable coexistence of phototrophic lineages from five bacterial phyla and present metagenome-assembled genomes (MAGs) of two uncultured *Chlorobiales* species. The MAGs contained genes involved in sulfur oxidation and photopigment biosynthesis, but also operons encoding for terminal oxidases. The metagenomes contained numerous contigs affiliating with *Microviridae* viruses, potentially affecting *Chlorobi*. Our data suggest a short sulfur cycle in which elemental sulfur produced by sulfide-oxidizing phototrophs is reduced back to sulfide by *Desulfuromonas* sp.

The release of sulfide creates a habitat selecting for anoxygenic sulfur-oxidizing phototrophs, which in turn create a niche for sulfur reducers. Strong syntrophism between these guilds seems to drive a short sulfur cycle that may explain the rapid development of the bloom. The high biomass yield of *Chlorobi*-affiliated organisms implies that the studied lineages thrive in the presence of oxygen, which is corroborated by oxidases found in their MAGs. The findings improve our understanding of the ecology and ecophysiology of anoxygenic phototrophs in hypoxic habitats and their impact on the coupled biogeochemical cycles of sulfur and carbon.

### 305-PMV

DNA-methylation is involved in the regulation of transcription, fine-tuning of DNA replication and DNA repair in the cyanobacterium *Synechocystis* sp. PCC 6803

\*M. Hagemann<sup>1</sup>, K. Gärtner<sup>1</sup>, S. Klähn<sup>2</sup>, I. Scholz<sup>3</sup>, S. Mikkat<sup>4</sup>, W. R. Hess<sup>3</sup>

<sup>1</sup>University of Rostock, Plant Physiology, Rostock, Germany

<sup>2</sup>UFZ, Department of Solar Materials, Leipzig, Germany

<sup>3</sup>University Freiburg, Genetics & Experimental Bioinformatics, Freiburg, Germany

<sup>4</sup>University Rostock, Core Facility Proteome Analysis, Rostock, Germany

DNA methylation plays a crucial role for gene regulation among eukaryotes, but its regulatory function is less documented in bacteria. In the cyanobacterium *Synechocystis* sp. PCC 6803 five DNA methyltransferases have been identified. Among them, M.Ssp6803II is responsible for the specific methylation of the first cytosine in the frequently occurring motif GGCC, leading to N4-methylcytosine (GGm4CC). The mutation of the corresponding gene *sll0729* led to lowered chlorophyll/phycoerythrin ratio and slower growth. Transcriptomics only showed altered expression of *sll0470* and *sll1526*, two genes encoding hypothetical proteins. Moreover, prolonged cultivation revealed instability of the initially obtained phenotype. Suppressor mutants appeared where the *sll0729* gene was still completely inactivated and the GGCC sites remained unmethylated. The suppressor strains showed smaller cell size, lowered DNA content per cell, and decreased tolerance against UV compared to wild type. Promoter assays revealed that the transcription of the *sll0470* gene was still stimulated in the suppressor clones. Proteomics identified decreased levels of DNA topoisomerase 4 subunit A in suppressor cells. Collectively, these results indicate that GGm4CC methylation is involved in the regulation of gene expression, in the fine-tuning of DNA replication, and DNA repair mechanisms.

### 306-PMV

Impact of c-di-GMP on the motor ATPase PilB1 of the type IV pilus in the model cyanobacterium *Synechocystis* sp. PCC 6803

\*T. Wallner<sup>1</sup>, H. Kuemmel<sup>1</sup>, C. van der Does<sup>2</sup>, A. Wilde<sup>1</sup>

<sup>1</sup>University of Freiburg, Institute of Biology III - Molecular Genetics, Freiburg, Germany

<sup>2</sup>University of Freiburg, Institute of Biology III - Molecular Biology of Archaea, Freiburg, Germany

Many cyanobacteria are able to move across surfaces using type IV pili thus, enabling the cells to make decisions about lifestyle and to actively search for suitable environmental conditions. Although light-controlled movement of cyanobacteria has been observed and described for a long time, the basic biophysical and molecular mechanisms are poorly understood. The model cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) shows light-induced motility on surfaces using type IV pili depending on the quantity and quality of light. Cyclic nucleotide second messengers such like c-di-GMP and cAMP are involved in the regulation of motility. However, the molecular basis how cyclic nucleotides affect this process are not well characterised.

The c-di-GMP binding domain MshEN domain was discovered in the motor ATPase MshE of *Vibrio cholerae* [1]. The MshEN domain is conserved in cyanobacterial proteins and can be found in PilB1, the type IV pilus secretion motor ATPase of *Synechocystis*, which is crucial for phototaxis. Interestingly, the MshEN domain is also found in PilB2 protein, another PilB homologue of *Synechocystis*, that is dispensable for motility. Using different strategies to optimize heterologous protein expression and affinity chromatography, we were able to purify the MshEN domain of PilB1 and PilB2, which were used in a fluorescence-based approach to verify binding of the c-di-GMP ligand to this domain. Further, we mutated several amino acids that constitute the binding pocket to prevent binding of c-di-GMP. These protein variants were used in our assay as well. In addition, we currently investigate if and how the altered binding of c-di-GMP to the PilB1 protein modifies the function

of the motor ATPase and in what way this changes the motility of *Synechocystis* cells.

1. Wang, Y.C., et al., *Nucleotide binding by the widespread high-affinity cyclic di-GMP receptor MshEN domain*. *Nat Commun*, 2016. **7**: p. 12481.

---

### 307-PMV

#### The nitrogen-regulated gene *ssr0692* encodes a small protein involved in controlling arginine metabolism in cyanobacteria

\*P. Bolay<sup>1</sup>, S. Timm<sup>2</sup>, M. Hagemann<sup>2</sup>, K. Forchhammer<sup>3</sup>, S. Klähn<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research, Department of Solar Materials, Leipzig, Germany

<sup>2</sup>University of Rostock, Department of Plant Physiology, Rostock, Germany

<sup>3</sup>University of Tübingen, Microbiology / Organismic Interactions, Tübingen, Germany

Cyanobacteria are the only bacteria performing oxygenic photosynthesis. Due to their lifestyle they are found in every light-exposed habitat. Besides their huge environmental impact (O<sub>2</sub> production, CO<sub>2</sub> fixation and primary production) they are of growing interest in biotechnology. However, knowledge of this bacterial clade is still limited, impeding the exploitation of their full biotechnological potential. E.g., knowledge of the regulation of cyanobacterial metabolism is scarce. Several examples indicate that cyanobacteria evolved a plethora of unique regulatory mechanisms governing metabolism. E.g., glutamine synthetase (GS) is controlled by the interaction with small proteins, so-called inactivating factors (IF) which are unique to but very common among cyanobacteria (for details see <sup>1</sup>). Moreover, cyanobacteria evolved unique RNA-based regulation to control the synthesis of these GS inhibitors <sup>2,3</sup>.

We identified a small protein that resembles the characterized IFs in several aspects. Gene homologs are present in most cyanobacteria, indicating an important function. Using the model strain *Synechocystis* sp. PCC 6803, we performed mutagenesis analysis of the corresponding gene *ssr0692* and metabolite profiling in response to changes in C/N balance. We observed striking differences, e.g. in the accumulation of arginine, a precursor of the cyanobacterial N storage compound cyanophycin. In WT, arginine spikes transiently when cells are suddenly exposed to high NH<sub>4</sub><sup>+</sup> levels. This was absent in strains with a constitutively high amount of *Ssr0692*. In contrast, *ssr0692* knockout strains featured a prolonged arginine accumulation compared to WT. Thus, these data suggest that, similar to the known IFs, a perfectly tuned synthesis of *Ssr0692* is crucial for controlling enzyme activity, likely in arginine metabolism. Hence, deploying small proteins as regulators of metabolic enzymes could be a general strategy in cyanobacteria.

1. Bolay, P. et al. *Life Basel Switz*. **8**, (2018).
2. Klähn, S. et al. *Proc. Natl. Acad. Sci. U. S. A.* **112**, E6243-6252 (2015).
3. Klähn, S. et al. *Nucleic Acids Res.* **46**, 10082–10094 (2018).

---

### 308-PMV

Is nitrogen fixation by *Nostoc* sp. PCC7524 increased in a simulated early earth and future atmosphere?

\*K. Ebel<sup>1</sup>, T. Bauersachs<sup>2</sup>, A. Hermann<sup>1</sup>, M. M. Gehringer<sup>1</sup>

<sup>1</sup>Technische Universität Kaiserslautern, Biology, Microbiology, Geomicrobiology, Kaiserslautern, Germany

<sup>2</sup>Christian-Albrechts-University, GeoChemistry, Kiel, Germany

The filamentous cyanobacterium, *Nostoc* sp. PCC7524, produces specialised cells known as heterocytes, which house the oxygen sensitive nitrogenase responsible for biological nitrogen fixation (BNF). The thick heterocyte cell wall reduces oxygen diffusion and contains specific glycolipids that are used as biomarkers for heterocystous cyanobacteria and BNF. Despite evidence of oxygen rich niches during the mid-Archean eon, there was no free oxygen in the Earth's atmosphere until the Great Oxidation Event (GOE), ~2.4 Ga. The delay in the expansion of early oxygenic phototrophs is thought to have been restricted by biologically accessible N. The aim of this project is to determine the effects of an oxygen free atmosphere and elevated CO<sub>2</sub> levels on BNF, photosynthesis and heterocyte glycolipid ratios. As climate change leads to elevated CO<sub>2</sub> in the present atmosphere, the understanding of the past may allow the prediction of changes in BNF, photosynthesis and glycolipid contents in heterocystous cyanobacteria.

*Nostoc* sp. PCC7524 was acclimatised to three different atmospheres under identical growth conditions: present atmospheric levels of O<sub>2</sub> and CO<sub>2</sub> (PAL), elevated CO<sub>2</sub> conditions (eCO<sub>2</sub>) and an Archean atmosphere without O<sub>2</sub> at eCO<sub>2</sub>. Differences in expression of the nitrogenase *nifD* and Rubisco *rbcl1* genes were measured via qRT-PCR and compared to active BNF (using the acetylene reduction assay) and photosynthetic rates (O<sub>2</sub> generation). Additionally, the heterocyte glycolipid, glycogen and protein content of end point cultures were analysed.

Our results showed, increased photosynthetic rates under eCO<sub>2</sub> conditions, while BNF rates were not substantially raised. Growth rates of *Nostoc* sp. PCC7524 grown under an Archean atmosphere were significantly reduced. Heterocyte glycolipids were present in the differently grown cultures, supporting their use as biomarkers of BNF. Data generated in this study will offer greater insight into BNF in heterocystous cyanobacteria under future (eCO<sub>2</sub>) and past (Archean) atmospheric conditions. Additionally, our study provides a novel view into the delay in the expansion of early cyanobacteria prior to the GOE.

---

### 309-PMV

Cyanobacterial whole cell biocatalysts efficiently utilize photosynthetic electrons for oxyfunctionalisation reactions

\*J. Toepel<sup>1</sup>, S. Lupacchini<sup>1</sup>, A. Hoschek<sup>1</sup>, M. Grund<sup>1</sup>, A. Schmid<sup>1</sup>, B. Bühler<sup>1</sup>

<sup>1</sup>UFZ Leipzig, SOMA, Leipzig, Germany

Whole-cell redox biocatalysis recently has been realized in a photosynthesis-driven way within cyanobacteria. Phototrophic host organisms possess the advantage to provide both co-substrates of oxygenases, O<sub>2</sub> and activated electrons, via the light-driven photosynthetic water splitting reaction. Thereby, oxygen mass transfer limitation, a major bottleneck in O<sub>2</sub>-dependent biocatalysis, can be avoided. We present results on the application of different oxygenases for photosynthesis-driven oxyfunctionalization in the photoautotrophic cyanobacterium *Synechocystis* sp. PCC6803. The strain containing a CYP450 oxygenase converted cyclohexane into cyclohexanol. In the absence of external O<sub>2</sub>, the reaction was shown to be light-dependent, demonstrating that photosynthetically generated O<sub>2</sub> is efficiently utilized for oxyfunctionalization. Quantitative physiological analyses of the photosynthetic apparatus allowed the identification of the potential of cyanobacterial strains to be applied for redox biocatalysis. Comparative analyses with a second oxygenase (a BVMO) also coupled to the photosynthetic apparatus gives us insight into the complex interplay of phototrophic metabolism and heterologous enzyme operation. We analyzed enzyme

activity, product formation and photosynthetic performance. The results showed that the photosynthetic electron flow rate is enhanced under specific reaction conditions to provide electrons for biocatalysis without reducing carbon fixation rates. The results of this study provide a strong and promising basis for the future exploitation of photosynthesis for productive biocatalytic oxyfunctionalization and redox biocatalysis in general.

Hoschek, A., Toepel, J., Hochkeppel, A., Karande, R., Bühler, B. and Schmid, A. (2019) Light-Dependent and Aeration-Independent Gram-Scale Hydroxylation of Cyclohexane to Cyclohexanol by CYP450 Harboring *Synechocystis* sp. PCC 6803. *Biotechnology journal*, e1800724.

### 310-PMV

Flavodiiron proteins as potential targets to improve photosynthetically-driven biotransformations in

*Synechocystis* sp. PCC 6803

\*L. Assil-Companion<sup>1</sup>, H. Büchsenschütz<sup>1</sup>, Y. Allahverdiyeva-Rinne<sup>2</sup>, M. Nowaczyk<sup>3</sup>, R. Kourist<sup>1</sup>

<sup>1</sup>Institute for Molecular Biotechnology, TU Graz, Graz, Austria

<sup>2</sup>University of Turku, Molecular Plant Biology, Department of Biochemistry, Turku, Finland

<sup>3</sup>Ruhr University Bochum, Faculty of Biology and Biotechnology, Bochum, Germany

In recent years, the potential of the photosynthetic microorganism *Synechocystis* PCC 6803 as a sustainable cellular factory for the production of various fine and bulk chemicals has come closer to fruition due to the development of various genetic tools<sup>1</sup> and a deeper understanding of its physiology. In particular, the photosynthetic electron transfer chain (PET) endows large amounts of NADPH which can be exploited for biocatalytic applications of heterologous NADPH-dependent enzymes. However, NADPH is also cellularly valuable; whereby various regulatory, protection and acclimation mechanisms make use of it.<sup>2</sup>

In particular, some flavodiiron proteins (Flv1 and Flv3), are considered to be NADPH: oxygen oxidoreductases that are actively involved in the protection of the photosynthetic apparatus and permit growth under fluctuating light.<sup>3</sup> In this work, we investigated the effects of a flavodiiron knock-out mutant ( $\Delta flv1$ ) on the catalytic activity of a heterologously expressed NADPH dependent ene-reductase (YqjM). We postulated that the deletion of flavodiiron proteins can lead to a higher NADPH availability within the cell without incurring significantly visible metabolic damage under controlled conditions.

We followed an *in vivo* substrate-in-product-out approach whereby cells were fed with 10 mM of substrate and the progress of the reaction was tracked over time. We report that following this approach, the deletion of Flv1 is beneficial for the enzymatic activity of YqjM whereby a significant improvement in specific activity is noted compared to the wildtype with conversions of > 99%. Additionally, we observed that different conditions during cultivation and biotransformation such as the cell density exerted a strong effect on the cellular activity.

### References

1. Liu, D. and Pakrasi, H.B., **2018**, *Microbial Cell Factories*, 17 (48), doi: 10.1186/s12934-018-0897-8

2. Holland, S.C., **2015**, *Biochimica et Biophysica Acta*, 1847, doi: 10.1016/j.bbabi.2014.12.001

3. Allahverdiyeva, Y. et.al, **2013**, *Proceedings of the National Academy of Sciences* 110 (10) doi: 10.1073/pnas.1221194110

### 311-PMV

Grad-Seq analysis of a photosynthetic bacterium rich in internal membrane systems

\*M. Riediger<sup>1</sup>, P. Spät<sup>2</sup>, B. Maček<sup>2</sup>, W. R. Hess<sup>1</sup>

<sup>1</sup>Albert-Ludwigs-Universität Universität Freiburg, Institute for Biology III, Genetics and Experimental Bioinformatics, Freiburg, Germany

<sup>2</sup>Eberhard Karls University Tübingen, Quantitative Proteomics, Tübingen, Germany

Compared to the well-studied RNA chaperones from enterobacterial model organisms, little is known about their functional counterparts in cyanobacteria, specifically those involved in posttranscriptional regulation via small non-coding RNAs (sRNAs). Homologues of those chaperones are either not conserved in cyanobacteria or showed no RNA binding activity (Bøggild et al., 2009 - *The FEBS Journal*, Schuergers et al., 2014 - *Mol. Microbiology*), suggesting the existence of yet non-described sRNA chaperones in cyanobacteria.

Gradient profiling by sequencing (Grad-Seq) was first established to identify novel RNA chaperones in the enterobacterial model organism *S. enterica* (Smirnov et al., 2016 - *PNAS*). This method provides a tool to infer the distribution of RNA-protein complexes along a sucrose density gradient, giving information on how RNAs and Proteins sediment within the gradient on a transcriptome- and proteome-wide scale.

We adapted this experimental approach for the cyanobacterial model organism *Synechocystis* sp. PCC6803, including an improved data analysis strategy to provide the first dataset of this kind for a photosynthetic organism. The co-fractionation analysis covers about 70% of the whole proteome and more than different 2500 transcripts and provides very promising clues not only into the distribution of RNA-protein complexes, but also for the deeper understanding of protein-protein and multi-protein interactions.

Particularly, the analysis of functionally related proteins that exhibit divergent gradient distributions or hypothetical proteins that are co-fractionating with well-annotated proteins allows for intriguing conclusions in various aspects of the cyanobacterial metabolism.

### 312-MTV

Controlling the bacterial type III secretion system through protein dynamics: Reversible dissociation of the cytosolic components of the type III secretion system at low pH prevents unregulated protein secretion during infection.

\*A. Diepold<sup>1</sup>

<sup>1</sup>Max Planck Institute for Terrestrial Microbiology, Ecophysiology, Marburg, Germany

To manipulate eukaryotic host cells, Gram negative bacteria can inject effector proteins via a type III secretion system (T3SS). We recently found that essential cytosolic components of the T3SS injectisome constantly shuttle between the structure and a cytosolic pool, and that this exchange is linked to the export of effectors.

We have now elucidated how bacteria benefit from this protein exchange, by using it to temporarily suppress and quickly reactivate T3SS secretion in response to external cues relevant during infection. The enteropathogen *Yersinia enterocolitica* uses its T3SS to invade Peyer's patches and fend off immune cells. Premature T3SS activation in the stomach could be detrimental. Fluorescence microscopy of live bacteria revealed that while core parts of the T3SS remain stable at low external pH, the essential cytosolic T3SS components rapidly dissociate to the cytosol. This reversible dissociation correlates with a transient suppression of T3SS function. Using single-particle tracking, we found that low external pH is sensed in the periplasm, and leads to a partial dissociation of a central ring structure in the inner membrane, which conveys the signal for the temporary dissociation of the cytosolic T3SS components. This striking phenotype allows bacteria to suppress secretion under unfavorable conditions, while enabling the fast initiation of secretion at the target site in the host. In support of this hypothesis, we found that assembly of the cytosolic T3SS components in *Pseudomonas aeruginosa*, which does not pass the stomach, is not pH-dependent.

Our results elucidate how bacteria can use protein dynamics to regulate the function of protein complexes such as the T3SS in response to external cues. Besides the molecular mechanism and its role in bacterial infection, I will present an application of this newly discovered regulatory principle: an engineered light-controlled T3SS that allows to precisely control bacterial protein secretion and translocation into host cells in space and time.

### 313-MTV

Structural and mechanistic insights into phospholipase A-mediated membrane phospholipid degradation related to the bacterial virulence

F. Bleffert<sup>1</sup>, J. Granzin<sup>2,3</sup>, M. Caliskan<sup>1</sup>, M. Siebers<sup>2</sup>, S. N. Schott-Verdugo<sup>4</sup>, B. Thiele<sup>5</sup>, L. Rahme<sup>6</sup>, P. Dörmann<sup>2</sup>, H. Gohlke<sup>4</sup>, R. Batra-Safferling<sup>3</sup>, K. E. Jaeger<sup>1,7</sup>, \*F. Kovacic<sup>1</sup>

<sup>1</sup>Heinrich Heine University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany

<sup>2</sup>Institute of Molecular Physiology and Biotechnology of Plants, University of Bonn, Bonn, Germany

<sup>3</sup>Forschungszentrum Jülich GmbH, Institute of Complex Systems - Structural Biochemistry (ICS-6), Jülich, Germany

<sup>4</sup>Heinrich-Heine-University of Düsseldorf, Institute of Pharmaceutical and Medicinal Chemistry, Düsseldorf, Germany

<sup>5</sup>Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, Plant Sciences (IBG-2) and Agrosphere (IBG-3), Jülich, Germany

<sup>6</sup>Harvard Medical School, Department of Microbiology and Immunobiology, Boston, Germany

<sup>7</sup>Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences (IBG-1), Jülich, Germany

Changes to the membrane involving degradation of membrane glycerophospholipids (GPLs), although fundamental for life, are poorly understood. Here, we describe PlaF, a cytoplasmic membrane-anchored phospholipase A of *Pseudomonas aeruginosa*, which modifies the membranes of this pathogenic bacterium by hydrolyzing endogenous GPLs. Fatty acids (FAs), released as products inhibit PlaF activity that is the hallmark of negative feedback regulation and underpin the role of PlaF in membrane turnover. We demonstrate that the hydrolytic activity of PlaF is regulated through reversible dimerization, which is dependent on FAs and protein concentration. The unique high-resolution crystal structure of the full-length dimeric PlaF with FAs bound to its active site reveals, for the first time, atomistic details of the homodimerization of single-pass TM proteins. Dimerization is mediated by interactions of the N-terminal transmembrane (TM) and, an unusually short, juxtamembrane (JM) domains that resemble a continuous

kinked  $\alpha$ -helix. The TM-JM helix allows constraining the active site cavity of monomeric but not dimeric PlaF, in direct contact with membrane PLs. FA-mediated interaction network which connects the catalytic and dimerization sites provides unprecedented structural details on the regulatory function of FAs on single-pass TM proteins. We propose a monomerization-triggered mode of activation of PlaF at the membrane surface by a TM-JM-dependent constraining mechanism. This mechanism, different from an allosteric response, is elicited by structural rearrangements of the membrane domains of single-pass TM proteins and might be conserved among a variety of enzymes with catalytic activity toward membrane-bound substrates. Virulence properties of PlaF observed in a *Drosophila melanogaster* infection model, the biofilm and swimming motility assays, pinpoint PlaF as the first target for the development of novel antibiotics interfering with PLA-mediated membrane GPL degradation.

### 314-MTV

Converting a bidirectional channel into an unidirectional channel – Studies on the translocation mechanism of *Escherichia coli* Formate Channel FocA

\*M. Kammel<sup>1</sup>, O. Trebbin<sup>1</sup>, G. Sawers<sup>1</sup>

<sup>1</sup>Martin Luther University Halle-Wittenberg, Microbiology, Halle a. d. S., Germany

Precise control of ion movements across biological membranes is of major importance for regulation of metabolism e.g. pH, energy or redox balance. Proteins performing these ion transport processes are divided into two general classes – ion channels and pumps (active transporters). While transporters move molecules against a concentration gradient, channels allow a molecule to move down its gradient. The formate channel FocA of *Escherichia coli* is a pentameric membrane protein of the formate-nitrate transporter (FNT) family. FNTs translocate monovalent anions across the cytoplasmic membrane of prokaryotic cells. FocA is the prototype of FNTs and it bidirectionally translocates formate, the key intermediate in mixed-acid fermentation of glucose in enterobacteria, across the membrane. Although, the translocation mechanism is not understood in detail, FocA is annotated as an ion channel. In the inward direction, passage of formate through FocA's hydrophobic pore is suggested to be facilitated by transient protonation via His209. This central histidine residue is conserved in more than 99% of FNT proteins and only a few pathogenic species have an FNT where the histidine is replaced by asparagine or glutamine.

Here we report on converting FocA into a unidirectional export channel by exchanging His209 to either Asn or Gln. Formate translocation by FocA variants was investigated using an *in vivo* reporter system based on a formate-inducible promoter. Studies on formate translocation into the cytoplasm were also performed using an *E. coli* strain lacking the formate-producing enzyme pyruvate formate-lyase (PflB), ensuring a low intracellular formate concentration. Our data revealed that FocA variants His209Asn and His209Gln displayed high formate efflux, even when cells were challenged with 20 mM exogenous formate. Surprisingly, formate import activity of these FocA variants was clearly detectable in strains lacking PflB. Our findings indicate that a protonatable histidine within the hydrophobic pore, as well as the cytoplasmic enzyme PflB both regulate gating of bidirectional formate translocation by FocA.

### 315-MTV

Structural analysis of two chemically driven Na<sup>+</sup>-translocating enzymes from anaerobic bacteria

\*S. Vitt<sup>1,2</sup>, S. Prinz<sup>2</sup>, U. Ermler<sup>2</sup>, W. Buckel<sup>1</sup>

<sup>1</sup>Philipps Universität, Fachbereich Biologie, Marburg, Germany

<sup>2</sup>Max-Planck-Institute für Biophysik, Frankfurt am Main, Germany

Strict anaerobic bacteria conserve energy via substrate-level phosphorylation and ion gradient forming processes. Examples of the latter are the Na<sup>+</sup>-translocating enzymes glutaconyl-CoA decarboxylase (Gcd) and ferredoxin: NAD reductase (Rnf). Here we describe our initial results of the structural characterization of these multisubunit membrane complexes by single particle cryo-electron microscopy (cryo-EM) and negative stain EM.

Gcd uses the free energy of decarboxylation ( $\Delta G^\circ = -30$  kJ/mol) of glutaconyl-CoA to crotonyl-CoA to drive 2 Na<sup>+</sup> from the cytoplasm through the membrane of the gut bacterium *Clostridium symbiosum* to generate  $\Delta\mu\text{Na}^+$  for ATP synthesis. Gcd is composed of 10 subunits, A<sub>4</sub>B<sub>2</sub>C<sub>1</sub>C<sub>2</sub>D<sub>2</sub>, of which A<sub>4</sub> catalyzes the transfer of the carboxylate of glutaconyl-CoA to biotin bound to C1 or C2. The decarboxylation of carboxybiotin mediated by subunits B drives sodium ion translocation, whereas subunits D may serve as membrane anchor of the A tetramer [1]. Cryo-EM of purified Gcd at 7 Å resolution revealed a soluble (A<sub>4</sub>) and a membrane-bound disc (B<sub>2</sub>+D<sub>2</sub>), which are connected by subunits C1 and C2 [2].

Rnf from the soil bacterium *Clostridium tetanomorphum* acts as ferredoxin:NAD reductase, which uses the reduction potential difference of ferredoxin (-420 mV) and NAD (-320 mV) for Na<sup>+</sup> pumping. In the reverse direction, driven by  $\Delta\mu\text{Na}^+$ , it enables formation of H<sub>2</sub> or fixation of N<sub>2</sub> with NADH as reductant. Rnf (172 kDa) was purified from the solubilized membrane fraction and consists of six different subunits A, B, C, D, E and G. RnfD and RnfG host covalently bound FMN and riboflavin, respectively. RnfB contains five iron-sulfur clusters and the ferredoxin binding-site. RnfC, carries two ferredoxin-like [4Fe4S]-clusters, one non-covalently bound FMN and a NADH binding site. RnfADGE shares high sequence similarity with the corresponding subunits of Nqr from *Vibrio cholerae* of which a crystal structure is known [3]. Until to date a preliminary negative-stain EM model of Rnf gives a rough idea how the subunits are arranged.

1. Kress et al. (2009) J Biol Chem 284, 28401-28409.
2. Vitt, Prinz, Ermler, and Buckel, submitted.
3. Steuber et al. (2014) Nature 516, 62-67.

### 316-MTV

#### MpsAB is important for *Staphylococcus aureus* virulence and growth at atmospheric CO<sub>2</sub> levels

\*S. H. Fan<sup>1</sup>, F. Goetz<sup>1</sup>

<sup>1</sup>Interfaculty Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbial Genetics, Tuebingen, Germany

Almost 50 years ago, carbon dioxide (CO<sub>2</sub>) dependent variants were described in non-autotrophic bacteria. However, the underlying mechanism of CO<sub>2</sub>-dependency are not clear. Here we show that the *Staphylococcus aureus* *mpsAB* (membrane potential-generating system) operon, known to play a role in membrane potential generation, is crucial for growth at atmospheric CO<sub>2</sub> levels. We constructed *mpsA*, *mpsB*, and *mpsABC* deletion mutants and studied their phenotypes. With the deletion mutants, we performed uptake studies with radiolabeled NaH<sup>14</sup>CO<sub>3</sub> (bicarbonate) and animal models of infection. Additionally, we complemented *Escherichia coli* carbonic anhydrase (CA) mutant which is unable to grow under atmospheric air but

can grow in the presence of high CO<sub>2</sub> with *S. aureus mpsAB* and vice versa. All deletion mutants hardly grew under ambient air conditions but could be restored to near wild-type levels under elevated CO<sub>2</sub> or bicarbonate concentrations. Uptake studies with radiolabeled NaH<sup>14</sup>CO<sub>3</sub> revealed that MpsAB represents a bicarbonate transporter. The *E. coli* CA mutant could be complemented by *Staphylococcus* specific *mpsAB*, and the growth of the *S. aureus mpsAB*(C) mutant could be complemented by the *E. coli* specific CA gene. *S. aureus mps* mutants produce less hemolytic toxin and are less virulent in animal models of infection. Homologs of *mmpA* and *mmpB* are widespread among bacteria, suggesting that they have a central metabolic function. We propose that MpsAB represents a dissolved inorganic carbon transporter, or bicarbonate concentrating system, possibly acting as a sodium bicarbonate cotransporter. This system plays an important role in fitness and pathogenicity and could explain the role of MpsAB beyond the known carbon dioxide-fixing bacteria.

Reference: Fan SH, Ebner P, Reichert S, Hertlein T, Zabel S, Lankapalli AK, Nieselt K, Ohlsen K, Gtz F., "MpsAB is important for *Staphylococcus aureus* virulence and growth at atmospheric CO<sub>2</sub> levels", *Nat Commun.* 2019 Aug 9;10(1):3627. doi: 0.1038/s41467-019-11547-5.

### 317-MTV

#### Biopearling of interconnected outer membrane vesicle chains by a marine flavobacterium

T. Fischer<sup>1</sup>, M. Schorb<sup>2</sup>, G. Reintjes<sup>1</sup>, A. Kolovou<sup>2</sup>, R. Santarella-Mellwig<sup>2</sup>, S. Markert<sup>3</sup>, E. Rhiel<sup>4</sup>, S. Littmann<sup>1</sup>, D. Becher<sup>5</sup>, T. Schweder<sup>3</sup>, \*J. Harder<sup>1</sup>

<sup>1</sup>Max Planck Institut for Marine Microbiology, Bremen, Germany

<sup>2</sup>EMBL Heidelberg, Electron Microscopy Core Facility, Heidelberg, Germany

<sup>3</sup>University of Greifswald, Dep. of Pharmaceutical Biotechnology, Greifswald, Germany

<sup>4</sup>Carl von Ossietzky University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Oldenburg, Germany

<sup>5</sup>University of Greifswald, Dept. for Microbial Proteomics, Greifswald, Germany

Outer membrane vesicles are well known, however, their biogenesis is not fully understood. Here we present evidence for a biological pearling of outer membrane vesicles.

Large surface-to-volume ratios provide optimal nutrient uptake conditions for small microorganisms in oligotrophic habitats. The surface area can be increased with appendages. Here we describe chains of interconnecting vesicles protruding from cells of flavobacterium strain Hel3\_A1\_48 affiliating with *Formosa* and originating from coastal free-living bacterioplankton. The chains were up to 10 µm long and had vesicles emanating from the outer membrane with a single membrane and a size of 80-100 nm by 50-80 nm. Cells extruded membrane tubes in the exponential phase, whereas vesicle chains dominated on cells in the stationary growth phase.

This formation is known as pearling, a physical morphogenic process in which membrane tubes protrude from liposomes and transform into chains of interconnected vesicles.

Proteomes of whole cell membranes and of detached vesicles were dominated by outer membrane proteins including the type IX secretion system and surface-attached peptidases, glycoside hydrolases and endonucleases. Fluorescein-labeled laminarin stained the cells and the vesicle chains. Thus, the appendages provide binding domains and degradative enzymes on their surfaces and probably storage volume in the vesicle lumen.

The flavobacterium is abundant during spring bacterioplankton blooms developing after algal blooms and has a special set of enzymes for laminarin, the major storage polysaccharides of microalgae. We demonstrated with fluorescently labelled laminarin that the vesicle chains bind laminarin or contain laminarin-derived compounds. Proteomic analyses revealed surface-attached degradative enzymes on the outer membrane vesicles. We conclude that the large surface area and the lumen of vesicle chains may contribute to the ecological success of this marine bacterium.

Fischer, T. *et al.* (2019) Biopearling of interconnected outer membrane vesicle chains by a marine flavobacterium. *Applied and Environmental Microbiology* 85, e00829-19

---

### 318-MTV

The author has not agreed to a publication.

---

### 319-MTV

#### High-resolution cryo-EM structures of respiratory complex I - mechanism, assembly and disease

\*K. Parey<sup>1,2</sup>, O. Haapanen<sup>3</sup>, V. Sharma<sup>4</sup>, H. Köfeler<sup>5</sup>, T. Züllig<sup>5</sup>, D. Mills<sup>1</sup>, J. Vonck<sup>1</sup>, W. Kühlbrandt<sup>1</sup>, V. Zickermann<sup>2</sup>

<sup>1</sup>Max Planck Institute of Biophysics, Department of Structural Biology, Frankfurt/Main, Germany

<sup>2</sup>Goethe University Frankfurt, Institute of Biochemistry II, Frankfurt/Main, Germany

<sup>3</sup>University of Helsinki, Department of Physics, Helsinki, Finland

<sup>4</sup>University of Helsinki, Institute of Biotechnology, Helsinki, Finland

<sup>5</sup>Medical University of Graz, Core Facility Mass Spectrometry, Graz, Austria

NADH:ubiquinone oxidoreductase (complex I) plays a key role in aerobic energy metabolism and is one of the largest membrane protein complexes known. Redox-linked proton translocation by complex I generates 40% of the proton motive force driving ATP synthase (1,2). Complex I dysfunction is associated with severe human diseases (3). Assembly of the 1 MDa complex I in the inner mitochondrial membrane requires assembly factors and chaperones. We have determined the structure of complex I from the aerobic yeast *Yarrowia lipolytica* by electron cryo-microscopy at 3.2 Å resolution (4). A ubiquinone molecule was identified in the access path to the active site. The cryo-EM structure indicated an unusual lipid-protein arrangement at the junction of membrane and matrix arms that was confirmed by molecular simulations. The structure of a complex I mutant and an assembly intermediate provide detailed molecular insights into the cause of a hereditary complex I-linked disease and complex I assembly in the inner mitochondrial membrane (4).

1. C. Wirth, U. Brandt, C. Hunte, V. Zickermann, *Biochim. Biophys. Acta* 1857, 902 (2016).

2. J. Hirst, *Annu. Rev. Biochem.* 82, 551 (2013).

3. R. J. Rodenburg, *Biochim. Biophys. Acta* 1857, 938 (2016).

4. K. Parey, O. Haapanen, V. Sharma, H. Köfeler, T. Züllig, S. Prinz, K. Siegmund, I. Wittig, D. J. Mills, J. Vonck, W. Kühlbrandt, V. Zickermann, *Sci. Adv.*, in press (2019).

---

### 001-HYP

#### Whole-genome analysis of vancomycin-resistant *Enterococcus faecium* (VREfm) causing nosocomial outbreaks suggests the occurrence of few endemic clonal lineages in Bavaria, Germany

\*D. Eisenberger<sup>1</sup>, C. Tuschak<sup>1</sup>, S. Nickel<sup>1</sup>, V. Lehner-Reindl<sup>1</sup>, C.

Höller<sup>2</sup>, B. Liebl<sup>2</sup>, G. Valenza<sup>1</sup>

<sup>1</sup>Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Erlangen, Germany

<sup>2</sup>Bavarian Health and Food Safety Authority, Hygiene, Oberschleißheim, Germany

#### Question

Among clinical isolates of Vancomycin-resistant *Enterococcus faecium* (VREfm), sequence type (ST) 117 and ST80 are highly prevalent in German hospitals and represent a frequent cause of nosocomial outbreaks. In this study, we investigated the genetic diversity of clinical isolates of VREfm recovered from different nosocomial outbreaks in Bavaria, Germany, by whole-genome sequencing (WGS).

#### Methods

Between January 2018 and April 2019, 100 non-replicate clinical isolates of VREfm originating from nosocomial outbreaks at eight different hospitals in Bavaria were investigated for genetic diversity by WGS using the Illumina MiSeq platform (Illumina Inc., San Diego, USA) and laboratory procedures according to the manufacturer's instructions. Afterwards, complex types (CTs) were identified by core genome multilocus sequence typing (cgMLST) using SeqSphere+ software version 6.0.2 (Ridom GmbH, Muenster, Germany). Furthermore, a single-nucleotide polymorphisms (SNP)-analysis was conducted for all VREfm strains using BioNumerics 7.6 software (Applied Maths, Sint-Martens-Latem, Belgium).

#### Results

Most of the isolates of this study (84%) belonged to three major clonal groups: ST80/CT1065like *vanB* (n = 45; 6 hospitals), ST117/CT71like *vanB* (n = 20; 5 hospitals) and ST78/CT894like *vanA* (n = 19; 3 hospitals). Isolates of the predominant lineage ST80/CT1065like *vanB* occurred in 6 different Bavarian hospitals and showed by SNP analysis a maximum difference of 34 SNPs. Forty-two Isolates of the above-mentioned lineage could be grouped into two subclusters (subcluster I: 28 genetically closely related isolates from 4 hospitals with a maximum difference of 13 SNPs and subcluster II: 14 genetically closely related isolates from 4 hospitals with a maximum difference of 12 SNPs).

#### Conclusion

Whole-genome analysis of VREfm causing nosocomial outbreaks suggests the occurrence of few endemic clonal lineages (ST80/CT1065like *vanB*, ST117/CT71like *vanB* and ST78/CT894like *vanA*) in Bavarian hospital settings.

---

### 002-HYP

#### Hijacking of host cellular function in *Staphylococcus aureus* infections

\*V. Behrends<sup>1</sup>

<sup>1</sup>University of Roehampton, Life Sciences, London, United Kingdom

**Introduction** - *Staphylococcus aureus* is one of the leading causes of hospital-acquired infections and a major human pathogen worldwide. As a facultative intracellular pathogen,

*S. aureus* is able to invade both phagocytic and non-phagocytic cells. The emergence of multidrug-resistant strains, such as methicillin-resistant *S. aureus* (MRSA) has made treating *S. aureus* infections significantly more complicated. Therefore, there is an urgent need to find novel therapies.

**Objectives** - This study aimed to identify and target host molecular pathways exploited by MRSA during intracellular infection with a view of identifying possible host-directed therapies.

**Methods** - We followed a three-pronged approach. (1) We used stable-isotope labelling coupled to state-of-art metabolomics techniques to investigate the impact of bacterial infection on host carbon metabolism. (2) We screened over one hundred FDA-approved host targeting drugs for their potential to increase host cell survival. (3) We used shRNA silencing to identify genes/gene product important for intracellular bacterial proliferation.

**Results** - We found extensive metabolic re-routing and detected several distinct metabolic changes that suggested starvation-induced autophagy in infected cells. We identified the molecular cascade activated in our infection model and our data suggested that *S. aureus* induces the autophagic flux during cell invasion for ATP generation and nutrient scavenging. Accordingly, drug-induced inhibition of AMPK, the responsible host kinase, hampered *S. aureus* intracellular proliferation. Further, from the results of our drug screen we identified three potentially re-purposable tyrosine kinase inhibitors that act via an EPHA2-mediated mechanism. Finally, our shRNA screen identified the host factor Tram2 as a novel factor important for intracellular staphylococcal infection.

**Conclusion** - Our findings show that targeting host pathways essential for MRSA survival is a viable therapeutic strategy against this versatile pathogen.

---

### 003-HYP

Establishing links between environmental contamination and transmission of vancomycin-resistant Enterococci in the healthcare setting: a whole genome sequencing-based approach

\*H. Tönnies<sup>1</sup>, C. L. Correa-Martinez<sup>1</sup>, N. J. Froböse<sup>2</sup>, A. Mellmann<sup>1</sup>, S. Kampmeier<sup>1</sup>

<sup>1</sup>University Hospital Münster, Institute of Hygiene, Münster, Germany

<sup>2</sup>University Hospital Münster, Institute of Medical Microbiology, Münster, Germany

### Introduction

The incidence of vancomycin-resistant enterococci (VRE) infections considerably increased during the last decades. In hospitals, VRE are able to persist in the environment for several years; these environmental contaminations can serve as a reservoir for nosocomial infections. We hypothesize that the patient's environment is a relevant source of nosocomial VRE transmissions and that there is an increased risk of VRE acquisition for individuals assigned to rooms previously occupied by VRE-positive patients.

### Objectives

This study aims to analyze the role of the environmental contamination in the transmission of VRE by using whole genome sequencing (WGS)-based typing.

### Materials and Methods

Environmental samples were collected from 2013 to 2019 during infection control visits and sent to the microbiological laboratory for VRE detection. Confirmed VRE strains underwent WGS using Illumina sequencing technology. The resulting sequences were retrospectively compared with patient isolates that were detected during the study period. Using multilocus sequence typing (MLST) and core genome (cg) MLST, their genotypic relationships were determined. Strains differing in  $\leq 3$  cgMLST alleles were rated as closely related suggesting a nosocomial transmission.

### Results

In total, 53 environmental samples containing VRE were identified. Of these 11 harboured *vanA*, 42 *vanB* and MLST ST203 (67.9%), ST192 (15.1%) and ST117 (9.4%), respectively. Comparison using cgMLST revealed that 97 patient isolates were genetically related to environmental strains. Among these, 67 were *vanA*, 26 *vanB* and four *vanA* and *vanB* positive and exhibited MLST ST203 (40.2%), ST192 (23.7%) and ST117 (21.6%), respectively. Deeper analysis of cgMLST showed that 49 of the 53 environmental isolates were closely related to patient isolates obtained.

### Conclusion

Inanimate surfaces in the hospital environment serve as relevant reservoir and potential source of transmission of VRE. Improving hand hygiene compliance to prevent environmental VRE contamination and optimising cleaning procedures of possibly contaminated surfaces is an important aspect in preventing nosocomial VRE transmissions.

---

### 004-HYP

Unraveling a severe Q-fever Outbreak in Kosovo March-April 2016: Rapid Deployment of an Outbreak Investigation Team

\*K. Stoecker<sup>1</sup>, K. Roßmann<sup>2</sup>, T. Morwinsky<sup>2</sup>, I. Fengler<sup>2</sup>, L. Zöller<sup>1</sup>, G. Genzel<sup>1</sup>

<sup>1</sup>Institut für Mikrobiologie der Bundeswehr, Abteilung für medizinische B-Aufklärung und Verifikation, München, Germany

<sup>2</sup>Bundeswehr Central Medical Service, Munich, Germany

Between March and April, 64 deployed soldiers of the multinational contingent in Prizren, South-Kosovo presented themselves at the military field hospital with severe headache, fever and respiratory tract symptoms. An atypical pneumonia was diagnosed in 43 of these patients; pulmonary infiltrates were confirmed by chest x-ray. Differential diagnostic tests for pathogens causing ambulant acquired pneumonias, carried out in the microbiological routine laboratory attached to the field hospital, were negative. Q-fever is known to be endemic in this area, but did not cause an outbreak in this extent in the course of the last years. A rapidly deployable outbreak investigation team (RDOIT) including a diagnostic laboratory capacity was deployed within 48 hours in order to support the field hospitals diagnostic capacities. Its mission was to confirm *Coxiella burnetii* as a potential causative agent of the current outbreak with molecular and serological diagnostics tests and epidemiological expertise. Overall, 64 clinical cases were investigated. Serum was obtained from all patients, and swabs were taken from sheep flock which was grazed in close distance to the field camp. Overall, 64 patients with symptoms consistent with Q-fever were tested for the presence of DNA of *C. burnetii* and antibodies against the bacterium in the deployed field laboratory. In total, Q-Fever was confirmed in 43 patients. The probable source of the



infection could be confirmed by molecular analyses of vaginal swabs and feces of a nearby sheep flock. This mission was organized and conducted within 48 hours and proofed the RDOIT concept as a strong tool for outbreak management.

### 005-HYP

Rifaximin resistance of *Clostridioides difficile* associated with *rpoB* alleles and MLST clades

J. Schwanbeck<sup>1</sup>, T. Riedel<sup>2</sup>, J. Overmann<sup>2</sup>, U. Groß<sup>3</sup>, B. Wolfgang<sup>3</sup>, \*A. E. Zautner<sup>1</sup>

<sup>1</sup>Universitätsmedizin Göttingen, Medizinische Mikrobiologie, Göttingen, Germany

<sup>2</sup>DSMZ, Braunschweig, Germany

<sup>3</sup>University Medical Center Göttingen, Medical Microbiology, Göttingen, Germany

### Introduction

*Clostridioides difficile* is the most prevalent cause of nosocomial diarrhea and infectious enteritis. Rifaximin (RFX) was recently proposed as a possible alternative therapy option for *Clostridioides difficile* infection (CDI), especially in recurrent cases.

### Objectives

The aim of the study was to perform a survey regarding RFX susceptibility in a *C. difficile* test cohort that was representative for five clinically relevant clades. At a MIC [RFX] > 32 µg/mL the *rpoB* gene was sequenced to determine the underlying resistance alleles. All RFX-resistant isolates were whole genome sequenced to determine their phylogenetic relatedness.

### Materials & Methods

Agar dilution assays were used to determine RFX MICs of 136 clinical *C. difficile* isolates from Germany (86), Indonesia (29) and Ghana (21). Whole genome sequence data were obtained by a combination of single-molecule real-time (SMRT) and Illumina sequencing technology in addition to amplicon sequencing of *rpoB* genes. Ribotypes and MLST-STs were deduced from the whole genome sequences.

### Results

The epidemiological cut off of RFX was determined at a maximum MIC of 0.5 µg/mL while RFX resistance was determined at a MIC > 32 µg/mL. 10.3% (14/136) of the isolates was found to be resistant against RFX. The resistance rate is distributed among the individual regions as follows 4.7% (4/86) for Germany, 27.6% (8/29) for Indonesia, and 9.5% (2/21) for Ghana. Three different *rpoB* alleles could be associated with RFX resistance. One of them had not been described before. The presence of a specific *rpoB* allele correlates with the MLST clade of the isolate, i.e. the RFX-resistant isolates belonged to the MLST STs 1,2, and 4.

### Conclusion

(i) The overall RFX resistance rate in our test cohort was 10.3% due to the significant increased RFX resistant rate of Indonesian isolates of 27.6%. This may be due to the use of rifampicin e.g. in the treatment of tuberculosis in Indonesia.

(ii) RFX resistance could be associated with three MLST STs.

(iii) We were able to identify one novel *rpoB* allele.

### 006-HYP

Horizontal Transfer of Carbapenemase-Encoding Plasmids from *Enterobacteriaceae* to *P. aeruginosa*

\*K. Kocer<sup>1</sup>, S. Boutin<sup>1</sup>, K. Heeg<sup>1</sup>, D. Nurjadi<sup>1</sup>

<sup>1</sup>Universitätsklinikum Heidelberg, Heidelberg, Germany

### Introduction

The emergence of carbapenemase-producing *Pseudomonas aeruginosa* is a major global concern. Resistance may occur through genetic mutations or acquisition of transferable plasmids. While some carbapenemase-encoding plasmids are confined to certain species, broad range plasmids may be transferred across species.

### Objectives

In this study, we looked at various carbapenemase-bearing plasmids, if and under which condition a natural plasmid transfer between *Enterobacteriaceae* and *P. aeruginosa* can occur.

### Method

Conjugative transfer was performed in vitro by liquid mating using susceptible PAO-1 and PA14 *P. aeruginosa* strains as recipient strains. Donor strains were clinical isolates of *Enterobacteriaceae* carrying various carbapenemase genes (*bla*VIM, *bla*OXA-48, *bla*NDM and *bla*KPC) on plasmids of different incompatibility types isolated from patients with in vivo carbapenemase transfer between their different *Enterobacteriaceae* species. To investigate the impact of quorum sensing inhibition, liquid mating experiments were performed with or without addition of macrolides. Transconjugants were selected using a meropenem-supplemented selective medium. Whole genome sequencing was performed to confirm conjugative transfer and to investigate transferred genomic content by comparative genomic analysis.

### Results

Our results showed that the natural transfer of *bla*VIM- and *bla*KPC-encoding plasmids to *P. aeruginosa* was successful from two donors isolated from different patients. No difference was observed in the presence and absence of erythromycin. Alignment of the assembled draft genome and plasmid content of the donors, recipients and transconjugants confirmed horizontal plasmid transfer.

### Conclusion

Overall, the results demonstrate the potential for the dissemination of integron-encoded carbapenemase from *Enterobacteriaceae* to *P. aeruginosa* by natural plasmid conjugation without manipulation or inhibition of quorum sensing. Moreover, the horizontal transfer from *Enterobacteriaceae* might be the source of recently emerging KPC carbapenemase-producing *P. aeruginosa*.

### 007-HYP

Compliance in German acute care hospitals over 4 years. A longitudinal study with data from the national surveillance system HAND-KISS.

\*T. Kramer<sup>1,2,3</sup>, K. Bunte<sup>1,2,3</sup>, C. Schröder<sup>1,3</sup>, M. Behnke<sup>1,3</sup>, C. Reichhardt<sup>1,2</sup>, P. Gastmeier<sup>1,2,3</sup>, J. Walther<sup>1,2,3</sup>  
<sup>1</sup>Charité Universitätsmedizin Berlin, Hygiene und Umweltmedizin, Berlin, Germany  
<sup>2</sup>Aktion Saubere Hände, Berlin, Germany  
<sup>3</sup>National reference center for the surveillance of HAI, Berlin, Germany

## Question

Hand hygiene plays a crucial role in the prevention of healthcare-associated infections and transmission of multidrug-resistant bacteria. In 2008 the campaign "Aktion Saubere Hände" (ASH) was launched in Germany, based on the World Health Organization's "Clean Care is Safer Care" initiative. Direct observation and feedback of the results are key components in the improvement of hand hygiene compliance. In 2014 a voluntary national surveillance electronic tool for the documentation of directly observed compliance to hand hygiene was introduced (HAND-KISS).

## Methods

Direct observation of compliance to hand hygiene is voluntarily performed in the participating hospitals by trained local staff according to adapted recommendations of the WHO. We evaluated wards that reported annually at least 150 hand hygiene opportunities (HHO) of hand hygiene per observation period from January 1st 2015 until December 31st 2018.

## Results

296,191 HHO observed on 278 wards in 98 hospitals were included into analysis. Overall mean compliance increased from 73% to 79% ( $p < .0001$ ). Compliance improved in nursing staff from 77% to 82% ( $p < .0001$ ), physicians from 68% to 74% ( $p < .0001$ ) and other healthcare workers from 65% to 73% ( $p < .0001$ ) respectively.

Compliance significantly increased for all individual moments of the WHO-model except moment 2 "before aseptic procedures".

Multivariable regression analysis revealed that risk factors that a compliant hand disinfection was performed were every moment except prior to aseptic tasks, nurses creating the opportunity and ICUs being the type of ward where it was performed.

## Conclusions

Overall compliance in German hospitals increased over time. To improve HH compliance "before aseptic procedures" appears to be especially difficult and should be addressed explicitly. Underlying reasons need to be the focus of future investigations.

## 008-HYP

### Self Reported Compliance to the 5 Moments of Hand Hygiene Reports from Three Tertiary Care Hospitals in Germany

\*T. Kramer<sup>1</sup>, T. Holzmann<sup>2</sup>, W. Schneider<sup>2</sup>, A. Ambrosch<sup>2,3</sup>, H. Niesall<sup>4</sup>, P. Gastmeier<sup>1</sup>, S. Diefenbacher<sup>5</sup>  
<sup>1</sup>Charité - University Medicine Berlin, Institute for hygiene and environmental medicine, Berlin, Germany  
<sup>2</sup>University hospital Regensburg, Department of infection control and infectious diseases, Regensburg, Germany

<sup>3</sup>Krankenhaus Barmherzige Brüder Regensburg, Institute for laboratory medicine, microbiology and infection control, Regensburg, Germany  
<sup>4</sup>BODE Chemie GmbH – a company of the HARTMANN GROUP, Bode Science Center, Hamburg, Germany  
<sup>5</sup>University Ulm, Department of social psychology, Ulm, Germany

## Question

How does self reported compliance correlate to directly observed compliance in health care workers that have been exposed to feedback of their hand hygiene performance.

## Methods

A questionnaire was designed to ask for compliance to the 5 moments of hand hygiene during daily

practice. Wards that finished direct observation of hand hygiene compliance within 8 months prior to

questioning were included. Healthcare workers from three tertiary care medical centers were questioned.

## Results

Data from 15 wards (n=4 ICUs ; n=1 IMC; n=1 4 regular wards) were included. Overall 186 HCW participated in the

survey. Their self reported compliance was 78.2% (IQR:79.5-81.25). 2570 moments were directly observed with median compliance

of 80% (IQR:67-84). Median alcoholic handrub consumption was 73.8ml/patient day (IQR: 41.3-127.55) in the year during direct

observation. Nine wards self-reported their average compliance rate within a 5% margin of the directly observed compliance, while 5

wards underestimated their performance by more than 5%, and one ward overestimated their compliance by more than 5%. Overall a significant positive correlation between self-reported and observed compliance was found ( $r = .549$ ,  $p = .034$ ).

## Conclusion

Self reported does correlate with directly observed compliance on a ward level in centers with an established feedback culture. This might offer additional information on awareness of HCW on the importance of hand hygiene.

## 009-HYP

Global transcriptome analysis of the emerging pathogen *Stenotrophomonas maltophilia* revealed a distinct subset of genes commonly expressed in biofilm cells

\*M. Gudzuhn<sup>1</sup>, I. Alio<sup>1,2</sup>, U. Mamat<sup>3</sup>, U. Schaible<sup>3</sup>, J. Steinmann<sup>4</sup>, D. Danso<sup>1</sup>, A. Poehlein<sup>5</sup>, D. Yero<sup>6</sup>, C. Vollstedt<sup>1</sup>, W. R. Streit<sup>1,2</sup>  
<sup>1</sup>University of Hamburg, Microbiology and Biotechnology, Hamburg, Germany

<sup>2</sup>University Hamburg, Department of Microbiology and Biotechnology, Hamburg, Germany

<sup>3</sup>Research Center Borstel, Cellular Microbiology, Borstel, Germany

<sup>4</sup>Universitätsinstitut der Paracelsus Medizinischen Privatuniversität, Institut für Klinikhygiene, Medizinische Mikrobiologie und Klinische Infektiologie, Nürnberg, Germany

*Stenotrophomonas maltophilia* is one of the most frequently isolated multidrug resistant opportunistic pathogens. It contributes to disease progression in cystic fibrosis patients and is found in wounds, in other infected tissues and on catheter surfaces.

The aim of this study is to identify the processes and genes involved in the biofilm formation of *S. maltophilia*. Therefore, we analyzed biofilm profiles of 300 clinical and environmental isolates. Additionally, we analyzed the 3D structure of 40 clinical isolates and did a global transcriptome analysis of eight clinical isolates grown under biofilm conditions. Our analyzes revealed a high strain specific heterogeneity in biofilm forming capability, biofilm structure, antibiotic tolerance and virulence of the isolates independent of their phylogenetic position within the genus (Steinmann, Front Microbiol. 2018; 9:806, Gröschel *et al.*, Nature communication, 2020 (in revision)). The transcriptome analysis in the eight strains identified a set of 106 shared and coexpressed genes among the top 250 most strongly expressed genes. Among the top 20 highly expressed genes were the alcohol dehydrogenase AdhP, the DNA competence protein ComEA, two copies of the Ax21 protein and the peptidoglycan-associated lipoprotein Pal. The involvement of ComEA and the Ax21 proteins in biofilm formation and maintenance were verified by laser scanning microscopy and microtiter plate assay of corresponding deletion mutants. Further physiological assays were conducted to verify the importance of AdhP in biofilm formation.

The transcriptome data generated in this study together with other phenotypic and metabolic data represent so far the largest data set on *S. maltophilia* biofilm. This study now lays the foundation for the identification of key genes involved in biofilm formation in *S. maltophilia* and for further development of strategies fighting *S. maltophilia* infections in clinical settings.

## 010-HYP

### Influence of porin deletions on $\beta$ -lactam resistance in *Enterobacter cloacae*

\*J. Schauer<sup>1</sup>, S. G. Gatermann<sup>1</sup>, N. Pfennigwerth<sup>1</sup>

<sup>1</sup>*Ruhr-University Bochum, Department for Medical Microbiology, Bochum, Germany*

## Introduction

The increasing incidence of carbapenem-resistant Enterobacterales leads to increased frequency of therapeutic failure and thus constitutes a serious threat for critically ill patients. Carbapenem resistance often results from production of carbapenemases but can also be the result of other resistance mechanisms such as PBP mutations, overexpression of chromosomally encoded  $\beta$ -lactamases, overexpression of efflux pumps and loss of porins. Only little is known about the influence of porin loss on  $\beta$ -lactam resistance in *Enterobacter cloacae*.

In this study, the role of the *E. cloacae* porins OmpF, OmpC and OmpD in  $\beta$ -lactam resistance was investigated by deleting the respective genes of the laboratory strain *E. cloacae* DSM 30054.

## Objectives

To investigate the role of the porins OmpF, OmpC and OmpD in  $\beta$ -lactam resistance in *Enterobacter cloacae* DSM 30054

## Material & methods

Porin genes *ompF*, *ompC* and *ompD* of *Enterobacter cloacae* DSM 30054 were deleted by introducing a resistance cassette into the regarding locus using the  $\lambda$ -Red recombination system. MICs for  $\beta$ -lactams of the wildtype and respective mutants were determined by broth microdilution and interpreted according to EUCAST. Growth experiments to evaluate the effect of porin deletion on bacterial fitness were performed by continuous monitoring of the cell densities.

## Results

Deletion of the porin genes *ompF*, *ompC* and *ompD* had only minor effects on  $\beta$ -lactam resistance in *Enterobacter cloacae* DSM 30054. In contrast to this, combined knockout of *ompF* and *ompC* as well as *ompF* and *ompD* had a greater effect on MICs especially on those of the cephalosporins. Only a combination of porin loss and expression of the chromosomal AmpC increased the MICs for the carbapenems, except for imipenem. Only deletion of *ompC* lead to a prolonged generation time indicating a fitness-cost.

## Conclusion

Here we describe that deletion of *ompF*, *ompC* and *ompD* had only minor effects on  $\beta$ -lactam resistance and that expression of chromosomal AmpC played a major role in  $\beta$ -lactam resistance.

## 011-HYP

Genome-based characterization of clinical *Salmonella enterica* producing extended-spectrum-beta-lactamases (ESBLs), Germany 2017-19

\*M. Pietsch<sup>1</sup>, S. Simon<sup>1</sup>, S. Banerji<sup>1</sup>, E. Trost<sup>1</sup>, Y. Pfeifer<sup>2</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>*Robert Koch Institute, Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*

<sup>2</sup>*Robert Koch Institute, Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany*

## Introduction

During the years 2017 - 2019 more than 45,000 human cases of Salmonellosis were reported in Germany, with around 15,000 cases each year. In about 25%, *Salmonella* strains were sent for further analysis to the German National Reference Centre for *Salmonella* and other Bacterial Enterics (NRC). Here we analyze the subset of 3<sup>rd</sup> generation cephalosporin resistant *Salmonella enterica* and report on the phylogenetic relationship and underlying resistance mechanisms in these isolates.

## Methods

For all received *S. enterica* serotyping and antimicrobial susceptibility testing was performed. The genomes of 269 isolates (excluding the serotypes *S. Typhi*, *S. Paratyphi ABC* and travel related isolates) with resistance to cefotaxime and/or ceftazidime was sequenced (Illumina, MiSeq).

Phylogenetic analyses, resistance gene detection and plasmid characterization were performed.

## Results

From 2017 to 2019 the NRC detected 269 clinical *S. enterica* isolates with resistance to 3<sup>rd</sup> gen. cephalosporins (2.17 % of all received *S. enterica*), with a modest increase from 1% 2017 up to 2% in 2019. Among the 269 isolates 35 different serovars were identified, with *S. Typhimurium* as the most prevalent one (n=104; 38.6%). However, 72 of these isolates were attributed to one single regional event and clustered phylogenetically. Hence, *S. Infantis* was the most prevalent serotype (n=40). Preliminary genome analyses exhibited the presence of ESBL gene *bla*<sub>CTX-M-1</sub> in the majority of the above mentioned isolates. Additional investigation of the *S. Typhimurium* isolates depicts a single, long time circulating clone, which acquired an approx. 200kb sized plasmid carrying *bla*<sub>CTX-M-1</sub> and the gene *qnrS1* contributing to fluoroquinolone resistance.

## Conclusion

The occurrence of 3<sup>rd</sup> gen. cephalosporin resistance in *S. enterica* of various serovars is most probably due to multiple acquisition events of conjugative plasmids. Further, our data imply the spread of a distinct cephalosporin-resistant *S. Typhimurium* strain in a regional setting.

## 012-HYP

Automated Email requests for MRSA screening of long-stay patients in a maximum-care hospital with low MRSA-prevalence are effective in detecting missed nosocomial transmissions

\*B. Tittmann<sup>1</sup>, Z. Bartha<sup>1</sup>, A. Dokic<sup>1</sup>, T. Juretzek<sup>1</sup>, M. Meyer<sup>1</sup>, M. Rabes<sup>2</sup>, H. Peltroche<sup>1</sup>

<sup>1</sup>Carl-Thiem-Klinikum, laboratory medicine, microbiology and hospital hygiene, Cottbus, Germany

<sup>2</sup>Carl-Thiem-Klinikum, medical informatics and documentation, Cottbus, Germany

## introduction

In Germany risk-adapted screening on admission for MRSA is recommended. The Carl Thiem Hospital (CTK) is a maximum care hospital in the federal state of Brandenburg with a low MRSA prevalence on admission (2018: 0.27 per 100 pts). In 2018, we additionally implemented MRSA screening for long-stay patients every three weeks. In order to increase compliance, an electronic Email request was automatically sent to the wards by the hospital information system on the day the screening was required.

## objectives

Our study aimed to evaluate compliance with the in-house screening algorithm and its effectiveness to detect MRSA transmission, thereby preventing further nosocomial MRSA spread.

## materials and methods

We analysed all Email requests sent between October 2018 and September 2019 and all laboratory screening orders for MRSA (NOT on admission) from the laboratory information system. Matching case numbers and dates of Email requests we determined the proportion and results of screenings carried out within 5 days, if the patient was not discharged within the following three days.

## results

In total 3,044 Email requests were sent with an average of 253 Emails per month. After 21 days 69.7% (n=2,121) of Emails were generated, after 42 days 15.4% (n=469), and 6.7% (205) after 63 days of hospitalization. Overall, 1,840 requests (60.4%) were included in the analysis. Screening was carried out in 53% (n=977) ranging from 86% (n=108) to 4% (n=1) per ward. Seven screening results were MRSA positive without prior detection of MRSA on admission to the hospital. One transmission event could be linked to a ward where members of staff were previously examined and discovered to be colonized with MRSA.

## conclusions

Although only a few MRSA colonisations were additionally detected by applying the novel screening algorithm, the procedure proved to be very effective in identifying nosocomial transmissions of MRSA that would otherwise have been missed and could probably cause outbreaks. Of special importance were wards with high risk patients and generally prolonged length-of-stay.

## 013-HYP

Trends of antibiotic consumption in German hospitals from 2015-2018

\*B. Schweickert<sup>1</sup>, M. Feig<sup>1</sup>, S. Marc<sup>1</sup>, G. Karin<sup>1</sup>, M. Behnke<sup>2</sup>, L. A. Pena Diaz<sup>2</sup>, P. Gastmeier<sup>2</sup>, M. Abu Sin<sup>1</sup>, D. Richter<sup>1</sup>, H. P. Blank<sup>1</sup>, H. Wehrmeyer<sup>1</sup>, A. Hoffmann<sup>1</sup>, T. Eckmanns<sup>1</sup>

<sup>1</sup>Robert Koch-Institut, Fachgebiet für nosokomiale Infektionen und Surveillance von Antibiotikaresistenz und Antibiotikaverbrauch, Berlin, Germany

<sup>2</sup>Institut für Hygiene und Umweltmedizin, Berlin, Germany

**Introduction:** The Antibiotic Consumption Surveillance (AVS)-system has been built up by the national public health institute in cooperation with the national reference center for surveillance of nosocomial infections in order to serve the hospitals in monitoring antimicrobial consumption and to support antibiotic stewardship activities.

**Objectives:** To analyze trends in antibiotic consumption over time.

**Methods:** The calculation of antibiotic consumption values is based on the ATC (Anatomical Therapeutic Chemical) /DDD (Defined Daily Dose) method of WHO (ATC/DDD-version 2019). Target value is the antibiotic consumption density (CD) expressed in DDD per 100 patient days (PD). The data of systemically applied antibiotics (A07AA, J01, J04AB02, P01AB01) are presented as medians (IQR). The analysis results rely on the data submission of general acute care hospitals from the years 2015-2018 and represent sequential cross-sectional analyses. Trend analysis was performed by linear regression.

**Results:** From 2015 to 2018 the number of participating hospitals increased from 103 to 209 hospitals. The median of total antibiotic consumption shows a decrease from 55.1 DDD/100 PD in 2015 to 51.2 DDD/100 PD in 2018. Analysis of the different antibiotic groups reveals a significant decline of the CD of cephalosporins from 17.4 DDD/100 PD to 12.1 DDD/100 PD, which is primarily due to a decrease of 2. generation cephalosporins while 3. generation cephalosporins remained nearly constant. A decrease can also be observed for macrolides, fluoroquinolones, imidazoles, sulfonamides/trimethoprim and glycopeptides. In contrast, the group of penicillins shows an increase from

14.0 DDD/100 PD to 16.2 DDD/100 PD which includes a rise of broad-spectrum as well as small-spectrum penicillins.

**Conclusion:** The surveillance data show a slight decrease of total antimicrobial consumption in German hospitals primarily driven by a decline of the use of 2. generation cephalosporins, which is partly compensated by an increase of penicillins. The data may indicate an effect of antibiotic stewardship activities and encourage for further enforcement and continuation of efforts.

#### 014-HYP

ARS Saxony: monitoring antibiotic resistance in Saxony

\*K. Flohrs<sup>1</sup>, E. Hennebach<sup>1</sup>, S. Wiegand<sup>1</sup>, W. Sydow<sup>1</sup>, I. Ehrhard<sup>1</sup>

<sup>1</sup>Landesuntersuchungsanstalt für das Gesundheits- und Veterinärwesen Sachsen, Medizinische Mikrobiologie und Hygiene, Dresden, Germany

**Introduction:** Increasing rates of critical antibiotic resistant organisms are a worldwide major health threat with regional distinct characteristics. In order to implement a comprehensive resistance surveillance in the Free State of Saxony, the Saxon Ministry of Social Affairs and Consumer Protection promoted the participation of Saxon microbiological laboratories in the national antimicrobial resistance surveillance system (ARS). All submitted data from Saxon institutions are taken in a Saxon data cube (ARS Saxony) for regional analyses.

**Objectives:** Resistance rates to selected antimicrobials were determined for microorganisms most frequently isolated in specimen from hospitals and the outpatient sector between 2015 and 2018.

**Materials & Methods:** We analysed routine data from 16 laboratories contributing to ARS Saxony. We included first isolates per patient and quarter in order to describe the spectrum of agents and resistance rates to selected antimicrobials. The prevalence of organisms with resistance to critical drugs and distribution by region and type of care was determined.

**Results:** We analysed resistance test information for 547.098 first isolates. Among the five organisms isolated most frequently in specimen from hospitals and the ambulant sector were *Escherichia coli* (25,6% and 36,2% respectively), *Staphylococcus aureus* (14,5% and 13,9%), *Klebsiella pneumoniae* (6,4% and 4,1%) and *Proteus mirabilis* (5,5% and 5,2%). MRSA rates were decreasing between 2015 and 2018. Rates of vancomycin-resistant *Enterococcus faecium* (VRE)-isolates from blood cultures were increasing significantly. The prevalence of MRSA and VRE revealed significant regional differences (lowest MRSA-rates and highest VRE-rates in the region of Leipzig). In 2018 resistance rates to cefotaxime were 11,0% in *E. coli* and 15,4% in *K. pneumoniae* isolates from hospitals as well as 7,0% and 7,4% respectively in the outpatient sector.

**Conclusion:** Data from ARS Saxony allow the surveillance of resistance rates in relevant microorganisms and the identification of regional characteristics.

#### 015-HYP

Joining forces to tackle resistant bacteria – a proposal for closer collaboration between public health authorities, physician scientists, and clinicians

\*B. Pantenburg<sup>1</sup>, A. Rother<sup>1</sup>, U. Helbig<sup>1</sup>, C. Riemschneider<sup>1</sup>, S. Walther<sup>1</sup>, I. Möller<sup>1</sup>, R. Krause-Döring<sup>1</sup>, N. Lahl<sup>1</sup>

<sup>1</sup>Gesundheitsamt Leipzig, Hygiene, Leipzig, Germany

**Introduction:** WHO lists antimicrobial resistance as a main threat to global health<sup>1</sup>. The One Health approach, based on interdisciplinary collaboration, aims at targeting this issue<sup>2</sup>. Similarly, the public health structure in Germany intends to intensify collaborations between public health authorities and the science sector<sup>3</sup>. However, public health authorities may be perceived ambiguously due to their varying tasks: from provision of counseling and support to supervision and sanctioning.

**Objective:** Taking MRSA outbreak management as an example, this abstract highlights what the public health department can add when joining forces with stakeholders such as clinicians and scientists.

**Material and Methods:** Analysis of MRSA outbreak records (8 episodes with  $\geq 2$  cases with an epidemiological link) in 4 hospitals in Leipzig (2010-2018).

**Results:** During outbreak management according to KRINKO guidelines<sup>4</sup>, the unique strengths of the public health department lie in: 1. Application of knowledge on emergency measures and prevention strategies gained in other settings to the current situation; 2. Providing the perspective of an "outsider", i.e. a "fresh" approach to existing issues; 3. Acting as a communication facilitator between stakeholders of different professions; 3. Use of the public health department's power based on the German Infection Protection Act to enforce measures in the stakeholders' common interest.

**Conclusion:** The public health department contributes valuable information during management of outbreaks with resistant bacteria. However, infrastructure for scientifically sound data analysis is scarce. Therefore, the public health department proposes a closer, and earlier, collaboration with clinicians and physician scientists: only in a joint approach major health challenges such as antibiotic resistance can be tackled.

#### References

1. <https://www.who.int/emergencies/ten-threats-to-global-health-in-2019>
2. <https://www.who.int/features/qa/one-health/en/>
3. <https://www.bvoegd.de/leitbild/>
4. Ausbruchmanagement und strukturiertes Vorgehen bei gehäuftem Auftreten nosokomialer Infektionen. Bundesgesundheitsbl-Gesundheitsforsch-Gesundheitsschutz 2002; 45:180-6

#### 016-HYP

**Isolation and characterization of bacteriophages specific for multidrug-resistant gram-negative bacteria**

J. J. Bugert<sup>1</sup>, R. Wölfel<sup>1</sup>, M. Ben Moussa<sup>2</sup>, S. Handrick<sup>1</sup>, S. Michniewski<sup>3</sup>, A. Millard<sup>4</sup>, C. Hinz<sup>1</sup>, C. Bugert<sup>1</sup>, D. Frieß<sup>1</sup>, F. Schwarze<sup>1</sup>, S. Mzoughi<sup>2</sup>, J. Kühn<sup>1</sup>, S. Kreissig<sup>1</sup>, M. Egold<sup>1</sup>, \*J. Stender<sup>1</sup>

<sup>1</sup>Institut für Mikrobiologie der Bundeswehr, Virologie, München, Germany

<sup>2</sup>Military Hospital of Tunis, Microbiological Laboratory, Tunis, Tunisia

<sup>3</sup>University of Warwick, Warwick Medical School, Warwick, United Kingdom

<sup>4</sup>University of Leicester, Department of Genetics and Genome Biology, Leicester, United Kingdom

The rise of multidrug-resistant gram-negative bacteria is a growing global problem which requires new therapeutic approaches. One alternative to antibiotic therapy is treatment with lytic bacteriophages. In Eastern Europe, and the US, bacteriophages are already used as efficient therapeutics. Regulatory issues regarding their safety and Good Manufacturing Practice (GMP) currently prevent their use in the EU. To address these issues, projects for establishing phage therapy, "Phage4cure" and "PhagoBurn", have obtained funding in Germany, France and Belgium. The Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures collects phages on behalf of these studies as a source for host specific phage cocktails.

In our laboratory, bacteriophages are isolated from surface/ sewage water and tested against a collection of multidrug-resistant gram-negative bacteria, including *Klebsiella* and *Escherichia* spp. A positive spot assay is followed by a plaque assay to isolate and purify the phage, produce a stock and determine the exact host range using high throughput methods (eg wzi capsule typing by PCR). Growth kinetics, using the TECAN Spark Instrument, are used to identify lytic phage efficacy. Suitable phages are further characterized by electron microscopy and genome sequencing. *Klebsiella* phage were successfully rebooted in *E. coli* using purified genomic DNA in our laboratory. This will allow the production of genetically modified phage with extended host range, eg to target multiple wzi capsule types of *Klebsiella pneumoniae*.

The Bundeswehr Institute for Microbiology uses modern phage technology to inform and support novel approaches in adjuvant phage therapy and as an important tool for the biological defense against natural or modified multidrug-resistant gram-negative bacteria.

## 017-HYP

Motivation, capabilities and opportunities as determinants of self-reported compliance with 26 surgical site infection-preventive measures among orthopaedic physicians: results of a "small sample with high response"-survey in a German tertiary care university hospital

\*I. Tomsic<sup>1</sup>, E. Ebadi<sup>2</sup>, F. Gossé<sup>3</sup>, I. Hartlep<sup>4</sup>, P. Schipper<sup>4</sup>, B. Schock<sup>4</sup>, I. F. Chaberny<sup>4</sup>, T. von Lengerke<sup>1</sup>

<sup>1</sup>Hannover Medical School, Centre of Public Health and Healthcare, Department of Medical Psychology, Hannover, Germany

<sup>2</sup>Hannover Medical School, Centre for Laboratory Medicine, Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany

<sup>3</sup>Clinic of Orthopaedics of Hannover Medical School at DIAKOVERE Annastift, Department of Spinal Surgery and Conservative Orthopaedics, Hannover, Germany

<sup>4</sup>Leipzig University Hospital, Institute of Hygiene, Hospital Hygiene and Environmental Medicine, Leipzig, Germany, Leipzig, Germany

**Question** Surgical site infection (SSI) prevention requires compliance with numerous measures [1]. Evidence regarding determinants of overall SSI-preventive compliance is lacking. Besides, orthopaedic physicians are an underresearched group. This study aims to identify associations between self-reported SSI-preventive compliance and determinants defined by the COM-B- (capability, opportunities, motivation and behaviour-) model [2].

**Methods** In a pretest to the WACH-trial (funding: Federal Ministry of Health, grant-ID: ANNIE2016-55-038; German Clinical Trials Register-ID: DRKS00015502), N=52 orthopaedic surgeons and anesthesiologists of an orthopaedic clinic participated in a questionnaire survey (response rate: 73.2%). Compliance regarding 26 SSI-

preventive measures and COM-determinants (18 items) were assessed by self-report. Factor analysis and linear regression models were used.

**Results** Factor analysis of capability and motivation items resulted in 3 factors (capabilities: 5 items/Cronbach's  $\alpha=0.89$ ; motivation: 4/0.83; and planning: 2/0.82), while 1 component emerged for environmental factors (opportunities: 6/0.87). Bivariate, self-reported compliance rate across all 26 measures (mean rate: 88.9%) was significantly associated with all factors. In contrast, only capabilities ( $\beta=0.42/p<0.01$ ) and planning ( $0.23/p<0.10$ ) showed positive effects in the final regression model.

**Conclusions** Planning of SSI-preventive measures may be a specific determinant of compliance, i.e. distinct from motivation and capabilities. Like interventions targeting hand hygiene [3], SSI-preventive implementation interventions should highlight healthcare worker's empowerment and planning skills.

## References:

[1] Commission for Hospital Hygiene and Infection Prevention (KRINKO) at the Robert Koch Institute. [Prevention of surgical site infections]. In German. Bundesgesundheitsbl 2018;61:448-73

[2] Michie S et al. The Behaviour Change Wheel. Implement Sci 2011;6:42

[3] von Lengerke T et al. Impact of Psychologically Tailored Hand Hygiene Interventions on Nosocomial Infections with Multidrug-Resistant Organisms. Antimicrob Resist Infect Control 2019;8:56

## 018-HYP

### Spread of OXA-244-producing *Escherichia coli* ST38 in Germany

\*J. B. Hans<sup>1</sup>, B. Neumann<sup>2</sup>, R. Kramer<sup>2</sup>, K. Kremer<sup>2</sup>, S. Haller<sup>2</sup>, N. Pfennigwerth<sup>1</sup>, A. Reuss<sup>2</sup>, Y. Pfeifer<sup>2</sup>, G. Werner<sup>2</sup>, T. Eckmanns<sup>2</sup>, S. Gatermann<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Abteilung für Medizinische Mikrobiologie, Bochum, Germany

<sup>2</sup>Robert Koch Institut, Berlin, Germany

Because of their enzyme-mediated antibiotic resistance, carbapenemase-producing Enterobacterales (CPE) have become a major threat for public health. Notifications of nosocomial infections and colonization with CPE are mandatory in Germany. Identification and characterization of carbapenemases is conducted at the National Centre for Multidrug-resistant Gram-negative Bacteria (NRC) through phenotypic and genotypic assays.

Between 2017 and 2018, numbers of OXA-244-producing *Escherichia coli* increased significantly. In 2019, case numbers continued to increase which prompted the Robert Koch Institute (RKI) to initiate an outbreak investigation.

From January 2017 until March 2019, a total of 145 OXA-244-producing *E. coli* isolates were identified, of which 92 isolates with known geographical origin were subjected to whole genome sequencing (Illumina). Analyses included multi-locus sequence typing (MLST), core genome (cg)MLST as well as high-resolution SNP analyses. Subsequently, a total of 60 additional isolates received between March and August 2019 were similarly processed.

Initial sequencing of 92 OXA-244-producing *E. coli* isolates identified a total of 14 different sequence types (ST) with a dominance of ST38 (n=60). Results of cgMLST and SNP analyses revealed close genetic relatedness of ST38 isolates, particularly 34 isolates formed a distinct cluster. However, detection of these 34 *E. coli* ST38 isolates was not geographically restricted but dispersed throughout the country. Furthermore, annually increasing numbers of isolates belonging to this cluster (2017: n=5; 2018: n=17; until 03/2019: n=12) suggest a continuing spread. Indeed, among the additionally 60 sequenced isolates, 28 isolates were identified to belong to this *E. coli* ST38 cluster. First detailed analyses suggest that the genomic localization of OXA-244 is diverse and associated with mobile genetic elements.

We here present a country-wide outbreak of OXA-244-CPE in Germany. However, further analyses are needed to identify routes of transmissions as well as the underlying mechanisms leading to an overall genetic homogeneity while permitting local plasticity of the OXA-244 gene.

---

## 019-HYP

### Control and molecular epidemiology of a Carbapenem non-susceptible *Klebsiella pneumoniae* cluster in hematologic patients

\*C. Baier<sup>1</sup>, F. Thol<sup>2</sup>, C. Koenecke<sup>2</sup>, F. C. Bange<sup>1</sup>, E. Ebadi<sup>1</sup>

<sup>1</sup>Hannover Medical School, Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany

<sup>2</sup>Hannover Medical School, Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover, Germany

## Introduction

Carbapenem resistance in Gram-negative bacteria (GNB) is an increasing threat for patients. Especially immunocompromised patients with hematologic diseases are at risk for colonization and infection.

## Objective

To describe the characteristics and control of a cluster with Carbapenem non-susceptible (C-NS) *Klebsiella pneumoniae* (Kp) on a hematologic ward.

## Materials/methods

The epidemiologic context was investigated by routine measures. Molecular strain analysis was performed with an in-house pulsed-field gel electrophoresis.

## Results

5 patients were found with rectal C-NS Kp colonization in 4 weeks exceeding the baseline level of C-NS GNB.

The index patient was transferred to the hematologic ward from an intensive care unit, where the screening for C-NS GNB took place on admission. The transfer took place before the positive result was available and the patient was not isolated initially. When the result became available (after 4 days), the patient was placed in a single room with strict barrier precautions

In addition, we started a weekly screening (rectal) for all patients on the ward (which is part of routine management when a patient with a C-NS Kp is hospitalized). The screening found successively 4 more patients with

nosocomial C-NS Kp. We suspected transmission and implemented the following measures: i) hand hygiene compliance monitoring, ii) training for healthcare workers iii) enhanced surface disinfection. The cluster was terminated and no further cases were found.

The molecular analysis showed that the cluster was caused by 3 different strains. The results suggest that three patients had the same strain (including the index). The other two patients had independent strains.

## Conclusion

The control measures together with increased awareness terminated the cluster without relapse. The prevalence screening enabled rapid implementation of isolation precautions for newly detected patients to interrupt transmission. Interestingly, the cluster was not monoclonal. Besides the index strain, there were two independent strains which may reflect selection phenomena in this patient population with frequent antibiotic use.

---

## 020-HYP

### Evaluation of clean room conditions for laboratory testing of sterile products using routine data on product and laboratory environment contamination

P. Dudler<sup>1,2</sup>, U. Vogel<sup>1,3</sup>, \*M. Hagemeister<sup>1</sup>

<sup>1</sup>Universitätsklinikum Würzburg, Stabsstelle Krankenhaushygiene, Würzburg, Germany

<sup>2</sup>Ludwig-Maximilians-Universität München, Institut für medizinische Informationsverarbeitung, Biometrie und Epidemiologie, München, Germany

<sup>3</sup>Universität Würzburg, Institut für Hygiene und Mikrobiologie, Würzburg, Germany

## Introduction

To ensure sterility of parenterally applied medications, the European Pharmacopoeia and the Guidelines of the EU for Good Manufacturing Practice (GMP), Annex 1, recommend principles of sterile pharmaceutical compounding. Lower grade clean areas than recommended for sterility testing might promote secondary contamination and lead to false-positive results. On the other hand, strict regulation of laboratories according to recommendations for manufacturers leads to higher costs possibly preventing the establishment of sufficient laboratory capacity.

## Objectives

This study describes the real world impact of limited structural clean room conditions on microbiological test results both for products and for laboratory environmental monitoring.

## Methods

Routine data from sterility tests of total parenteral nutrition (TPN) performed at a microbiological laboratory of a German university hospital from a five-year period were reviewed. Microbiological clean area monitoring data was assessed for compliance with recommended limits according to EU-GMP-Guidelines. Results of sterile filtration performed when clean room conditions complied with requested monitoring limits were compared to those when limits were exceeded.

## Results

In the observation period, 1617 of 1617 TPN were confirmed to be sterile. Microbiological monitoring despite limited structural conditions revealed grade "B" conditions in 290 of 333 monitoring days. We could not find a correlation between room conditions and results of sterile filtrations.

## Conclusion

According to our data, formal non-compliance with clean room grade recommendations of EU-GMP-Guidelines had no impact on the results of sterility testing in the observed laboratory. Organisational issues appear to be of greater importance to ensure the avoidance of false-positive results.

---

## 021-HYP

### Development of a Diagnostic Stewardship Assessment Framework for Antimicrobial Resistance (AMR) Surveillance in Low-Resource Settings

\*S. Tomczyk<sup>1</sup>, O. Ayobami<sup>1</sup>, A. Egwuenu<sup>2</sup>, T. Eckmanns<sup>1</sup>

<sup>1</sup>Robert Koch Institute, Berlin, Germany

<sup>2</sup>Nigeria Centre for Disease Control, Abuja, Nigeria

**Introduction:** The underutilization of diagnostics, particularly in low-resource settings, undermines the ability to perform antimicrobial resistance (AMR) surveillance and patient care. "Diagnostic stewardship" promotes coordinated guidance to improve the appropriate use of diagnostics along the pre- to post-analytic pathway.

**Objective:** The objective was to evaluate existing relevant tools and develop an evidence-based diagnostic stewardship assessment framework that could be used as a practical approach for evaluating needs and identifying priority activities particularly in low-resource settings.

**Materials & methods:** A literature review was conducted using PubMed, a manual search of relevant reference lists and expert personal communications. Identified guidance documents were compared, gaps were noted, Nigeria was assessed as a case study example, and themes were qualitatively summarized. The resulting assessment framework was subsequently validated in a pilot and consensus-based exercise.

**Results:** The diagnostic stewardship assessment framework was structured at the 1) health-system and 2) clinical level. At the system level, themes assessed were healthcare financing supporting patient access to diagnostics and investment in laboratory infrastructure and human capacity. A priority activity included the development of policy advocacy tools for universal coverage of blood culture diagnostics. At the clinical level, themes assessed were the pre-analytic (specimen collection, handling, transport, storage), analytic (laboratory processing, quality control), and post-analytic (data analysis, results feedback, communication methods between clinicians and microbiologists) phases. Priority activities included the local adaption of standard operating procedures to stimulate the appropriate use of blood cultures among suspected sepsis patients, initial procurement support and ongoing "bench-side" mentorship.

**Conclusion:** Financing of blood cultures and the development of practical stewardship tools are critical in settings such as Nigeria to support the basis for AMR surveillance. This assessment framework is a resource that could be locally adapted and validated.

## 022-HYP

### Screening clinical samples for linezolid-resistant enterococci

H. Claus<sup>1</sup>, C. Polzin<sup>1</sup>, \*U. Vogel<sup>1</sup>

<sup>1</sup>Institute for Hygiene and Microbiology, Würzburg, Germany

## Introduction

A recent recommendation of the German Commission for Hospital Hygiene and Infection Prevention (KRINKO) on hygiene procedures to prevent infections with enterococci emphasized the need to prevent further spread of linezolid resistance in enterococci. While screening for vancomycin-resistant enterococci (VRE) is well established in many hospitals, screening for linezolid-resistant enterococci (LRE) or linezolid-vancomycin-resistant enterococci (LVRE) is not available, but might be necessary for the management of increasing prevalence or outbreaks.

## Objective

Establishment of a routine procedure to screen for L(V)RE.

## Material and Methods

A selection of 21 clinical *Enterococcus* (E.) *faecium* isolates from the Würzburg strain collection (2 LRE, 11 LVRE, 8 VRE) was used for primary validation of culture media. The ongoing VRE screening programme at the University hospital of Würzburg provided de-identified samples to validate performance within the programme.

## Results

We defined the best suited linezolid concentrations in Enterococcosel™ broth and agar in the primary validation phase using clinical isolates. *E. gallinarum* and *E. casseliflavus* turned out to potentially grow in the media. These species give positive test results with MDG medium which can be used as a rapid test to exclude *E. faecium* or *faecalis* (Devriese et al., J. Clin. Micro. 1996). In order to include linezolid containing media in routine procedures, it was necessary to proof that the sensitivity of VRE detection by broth enrichment does not suffer from splitting samples. Analysis of more than 800 samples demonstrated that this is not the case. A workflow will be proposed.

## Conclusions

Linezolid containing enrichment broth and agar and a standardized workflow feasible for the clinical microbiology laboratory was established. The protocol enables to identify LRE and LVRE from anal and rectal swabs.

---

## 023-HYP

### Epidemiological investigation of KPC-producing *Klebsiella pneumoniae* in two different hospitals by Fourier Transform Infrared Spectroscopy (FTIRS)

\*M. Cordovana<sup>1</sup>, A. C. Veloo<sup>2</sup>, J. W. Rossen<sup>2</sup>, S. Ambretti<sup>1</sup>

<sup>1</sup>University Hospital Policlinico Sant'Orsola-Malpighi of Bologna, Microbiology, Bologna, Italy

<sup>2</sup>University of Groningen, University Medical Center Groningen, Department of Medical Microbiology and Infection Prevention, Groningen, Netherlands

## Background



KPC-producing *Klebsiella pneumoniae* (KPC-Kp) poses a serious infection control problem. The high-resolution molecular biology techniques required to track its spread are often unaffordable and laborious to perform. In this study, we applied FTIRS to investigate the epidemiological situation regarding KPC in the intensive care wards of two very different hospitals (a tertiary care university hospital and a long term rehabilitation facility). Well characterized isolates were included as a reference.

## Methods

Surveillance isolates collected from inpatients (n=48 for hospital A, n=25 for hospital B) during a 2 months time frame were analysed by the IR Biotyper system (IRBT, Bruker Daltonik). In addition, n=16 KPC-Kp strains with known ST data, derived from whole genome sequencing were added, to evaluate the discriminatory power of IRBT, and to design a possible model for typing. Infrared absorption spectra were acquired in transmission mode from a bacterial suspension in water/ethanol, placed in three replicates on a silicon sample plate, which were air dried. Using the IRBT software, spectra relation within a wavenumber range from 1300 to 800 nm was analysed applying hierarchical cluster analysis (HCA) with Euclidean metric and single linkage.

## Results

IRBT clustered the KPC-Kp isolates in 5 clusters in hospital A and 4 clusters in hospital B, respectively, and single/unique isolates. Comparison with the well characterized reference strains enabled to assign the IRBT clusters to the most common sequence types (ST-307, ST-101, ST-1519), with some significant differences in their distribution among the two hospitals. Further, several isolates from hospital A showed a relationship with the earlier sequence types (ST-258 and ST-512), while isolates from hospital B did not.

## Conclusions

In this study, FTIRS showed to be a promising method for epidemiological investigation for KPC-producing *K. pneumoniae*. Although further investigations are necessary to confirm and strengthen our findings, these first results suggest that IRBT could represent a reliable, fast and low-cost alternative typing method, suitable for routine screening in microbiology laboratory.

## 024-HYP

Process optimization and compliance with dressing change in pre-post intervention comparison after introduction of a new dressing change concept in the context of a prospective cohort study on infection prevention

\*R. Otchwemah<sup>1,2</sup>, M. Neuwirth<sup>1</sup>, B. Marche<sup>2</sup>, R. Galante<sup>1</sup>, S. Akca<sup>3</sup>, J. Hoffmann<sup>1</sup>, B. Bouillon<sup>4</sup>, F. Mattner<sup>1</sup>

<sup>1</sup>Clinics Cologne, Institute for Hygiene, Köln, Germany

<sup>2</sup>Cologne-Merheim Medical Center, Department of Trauma Surgery and Orthopedic Surgery, Köln, Germany

<sup>3</sup>University of Freiburg, Institute for nursing science, Freiburg i. Br., Germany

<sup>4</sup>Cologne-Merheim Medical Center, Department of Traumatology and Orthopedic Surgery, Cologne, Germany

## Introduction

The prospective cohort study "HygArzt" aims to demonstrate the effectiveness of infection prevention measures introduced by physicians responsible for hygiene. Therefore, the introduction of a new concept for dressing change and

compliance with hand hygiene were investigated in a level one orthopedic/trauma surgery center in a Germany.

## Materials & methods

The dressing change hand hygiene compliance was recorded with a modified checklist, based on the recommendations of the WHO. Four required hand disinfections were defined as indications: 1. "before preparation of the dressing change materials", 2. "immediately before dressing change", 3. "before clean phase", 4. "after dressing change". Based on the observed initial situation during the six months pre-intervention phase, a new dressing change concept was developed, which included additional equipment and measures to improve the dressing change process and support the compliance. The concept was implemented during a three months implementation phase and compliance to the concept and hand hygiene were reevaluated during a 6 months post-intervention phase. For statistical analyses the Chi-square tests was applied.

## Results

During the pre-intervention-, intervention- and post-intervention phases 331, 60 and 374 dressing changes were observed respectively. After the introduction of the new dressing change concept the hand hygiene compliance of all four indications improved highly significant ( $p < 0.001$ ). In the post-intervention phase the mean compliance showed values of at least 85%. Process parameters like usage of a clean work surfaces (pre: 13%; post: 36%), clean withdrawal of materials from the dressing trolley (pre: 7%; post: 77%) and appropriate waste disposal (pre: 4%; post: 39%) showed significantly increased compliance values after intervention as well ( $p < 0.001$ ).

## Conclusion

The introduction of the new dressing change concept, based on continuous process observation, has significantly increased the compliance to hand hygiene and further process parameters. The concept was successfully customized and introduced through theoretical and practical training by physicians responsible for hygiene.

## 025-HYP

**Negative pressure wound therapy in complicated spinal wounds: which impact do microbiological results have?**

\*J. Kellner<sup>1</sup>, S. Scheithauer<sup>2</sup>, A. Dudakova<sup>3</sup>, V. Rohde<sup>1</sup>, C. Von der Brölie<sup>1</sup>

<sup>1</sup>University Medical Center Goettingen, Neurosurgery, Goettingen, Germany

<sup>2</sup>University Medical Center Goettingen, Institute of Infection Control and Infectious Diseases, Goettingen, Germany

<sup>3</sup>University Medical Center Goettingen, Institute of Medical Microbiology, Goettingen, Germany

## Introduction

The incidence of surgical site infections (SSI) after complex spinal surgery is up to 20%. The impact of positive microbial results, above all changing ones in the course is not understood.

## Objective

This study should characterize the course of microbial colonization/infection during negative Pressure Wound

Therapy (NPWT) in order to identify potential risk factors for prolonged therapy.

## Materials and Methods

This is a retrospective observational study enrolling all patients after spinal surgery treated with NPWT. Outcome was analyzed categorizing therapy success (clinical and microbiological), length of hospital stay and number of NPWT procedures. Indication for wound closure was made clinically.

## Results

367 NPWT procedures in 82 patients with a mean of 4 consecutive NPWTs were analyzed.

56/82 (68%) patients showed typical pathogens of a wound infection (enterobacteria, non-fermenter, *S. aureus*, *Streptococcus* sp.) at least once. In 6/82 patients (7%) relevant bacteria changed during NPWT. Change was not associated with the number of NPWT (3/43, 7% <4 NPWT versus 3/39, 8%  $\geq 4$  NPWT;  $p=1.00$ ). Risk factors for multiple NPWTs were: multi drug resistant organisms (MDRO) in at least one swab (8/43, 19% <4 NPWT vs. 16/39, 41%  $\geq 4$  NPWT;  $p=0.031$ ) and at least one polymicrobial wound swab (17/43, 40% <4 NPWT vs. 25/39, 64%  $\geq 4$  NPWT;  $p=0.0298$ ). In 60% (49/82) wound closure was successful despite positive wound swabs. Thereof, in 14 (17%) patients MDRO were present. Both cases were not associated with mortality (cured 45/49, 92% vs. deceased 4/49, 8%;  $p=0.643$ ). Latest positive wound swab even with MDRO at wound closure was not associated with extended hospital stay (15/49, 31% >63 days vs. 34/49; 69%  $\leq 63$  days;  $p=0.102$ ).

**Conclusion** Bacterial contamination of NPWT wounds seems to be common, even with changing microbes and did not impact outcome. MDRO and polymicrobial swabs did impact outcome perhaps representing surrogates.

## 026-HYP

### Analysis of screening for multidrug-resistant bacteria of participating intensive care units of the EFFECT-study

J. Herrmann<sup>1</sup>, D. Hasenclever<sup>2</sup>, N. Köhler<sup>2</sup>, Y. Breitenstein<sup>3</sup>, A. Kühn<sup>1</sup>, \*I. F. Chaberny<sup>1</sup>

<sup>1</sup>University of Leipzig, Institute of Hygiene, Hospital Epidemiology and Environmental Medicine, Leipzig, Germany

<sup>2</sup>Medical Faculty of the University of Leipzig, Institute for Medical Informatics, Statistics and Epidemiology, Leipzig, Germany

<sup>3</sup>Medical Faculty of the University of Leipzig, Clinical Trial Centre, Leipzig, Germany

**Introduction and Objectives:** The ongoing EFFECT-study investigates whether daily antiseptic body wash with octenidine reduces nosocomial primary bacteraemia and nosocomial multidrug-resistant organisms in intensive care units (ICU). EFFECT-data allow describing the screening behavior for multidrug-resistant bacteria of all participating ICUs. In Germany, infection control guidelines of the Commission for Hospital Hygiene and Infection Prevention at the Robert Koch-Institute (KRINKO) exist for all multidrug resistant bacteria. However, the implementation of screening and contact precautions are very variable in clinical practice. **Materials&Methods:** Forty-four ICUs take part in EFFECT. The first ICU started the intervention in January 2017 and the last ICU in November 2018. At inclusion, we collected information on the current screening routine for multidrug-resistant bacteria at each ICU (screening behavior and contact-precautions of new patients), separately for

methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), and gram negatives bacteria with multidrug-resistant (MRGN). Results: For MRSA, MRGN and VRE, 31, 22 and 13 of 44 ICUs screen 100% of patients respectively. The remaining ICUs either conduct risk-based screening or do not screen at all. Contact-precautions are also very different. Conclusion: All ICUs implemented the infection control guidelines of the Commission for Hospital Hygiene and Infection Prevention correctly. Nevertheless, the screening behavior of the ICUs participating in the EFFECT-study is variable. Evidence based and workable guidelines are needed to standardized screening and implement good infection control practice.

## 027-HYP

### Hygiene & emotion: Enjoying work forms the basis for performance and success of hand hygiene

\*B. Schock<sup>1</sup>, I. Hartlep<sup>1</sup>, P. Schipper<sup>1</sup>, I. F. Chaberny<sup>1</sup>

<sup>1</sup>Leipzig University Hospital, Institute of Hygiene, Hospital Epidemiology and Environmental Medicine, Department of Diagnostics, Leipzig, Germany

Numerous intervention programs have been analyzed in various study designs. PSYGIENE has shown that tailored behavioral psychological intervention for medical staff leads to a lasting improvement in hand hygiene compliance [von Lengerke et al., 2017]. Innovative concepts enable promising results during the study period, but it often remains unclear how research impulses can be incorporated into hygiene routine. Therefore, a reality check was performed after four years of behavioral psychological infection control at Leipzig University Hospital. In addition, hand hygiene results should be included to assess success. A standardized interview for hygiene staff of the Institute of Hygiene, with a questionnaire on the benefits and acceptance of tailoring was conducted (5-Likert scale, 1 = fully up to 5 = does not apply at all). The ASH hand hygiene collection sheet is used for observation in 14 clinical areas (all ICU). Compliance has been monitored since 2015. Physicians and nurses are stratified. The results are reported to the employees in feedback sessions and analyzed together. The survey included all staff members (N= 15, physicians= 5). All employees were involved in the observations and feedback. Since 2015, over 17.000 hand hygiene opportunities were collected. Significant increase in hand hygiene compliance both in the ICU 2018: 76.3% (vs. 2015;  $p = 0.022$ ) and in the normal wards 78.8% ( $p < 0.001$ ) was observed. Almost all employees agree that they give helpful feedback. The benefit of tailored intervention is well-known (80%) and the employees enjoy working since they are convinced of the tailoring. The routine application of tailored interventions is associated with positive experiences and regular exchange in the team. Recent studies have been conducted about the insufficient implementation of infection control measures by the medical staff. In order to achieve a long-term success in infection control, the infection control team should be supported with regard to their attitudes and motivation for tailored intervention. The implementation of tailored intervention takes time to be implemented carried by the hospital staff as well as the infection control team.

## 028-HYP

### Wound infections and antibiotics consumption in surgery (WACH-trial): Guideline compliance in operating theatre (OT) – what's the current state?

\*P. Schipper<sup>1</sup>, B. Schock<sup>1</sup>, I. Hartlep<sup>1</sup>, I. Tomsic<sup>2</sup>, T. von Lengerke<sup>2</sup>, C. Krauth<sup>3</sup>, I. F. Chaberny<sup>1</sup>

<sup>1</sup>Leipzig University Hospital, Department of Diagnostics, Institute of Hygiene, Hospital Epidemiology and Environmental Medicine, Leipzig, Germany

<sup>2</sup>Hannover Medical School, Centre of Public Healthcare, Department

**Introduction:** The PSYGIENE-trial has shown that using custom behavioral psychology interventions to improve hand hygiene has a lasting effect within the group of healthcare professionals [von Lengerke et al., 2017]. After successfully improving hand hygiene in intensive care units at Hannover Medical School our goal was to evaluate further steps to prevent surgical site infections (SSI) in a separate trial.

**Objectives:** Preventing SSI requires compliance regarding many pre-, intra- and postoperative procedures. Therefore, perioperative procedures need to be investigated from an infection control point of view to answer the question, if the criteria for guideline-based preventive measures are met.

**Methods:** A compliance questionnaire for the OT personnel was developed based on KRINKO recommendations and AWMF guidelines (e.g. proper fitting of surgical caps and face masks), which was tested at Leipzig University Hospital. Overall, 54 standardized observations in the OTs were completed in a so-called Pre-WACH-phase. Patient-related as well as personnel-related characteristics, like wearing surgical masks correctly, were observed.

**Results:** The following results refer to the procedure in OTs. During 54 observations, a total of 243 employees were observed while working in daily routine. Just over half of them were wearing surgical caps correctly (compliance=53.1%; n=129). However, a higher compliance for wearing face masks correctly was observed (compliance=90.3%). In 7 out of 54 surgeries a clipper was used. No shavings were detected, which equals to a compliance of 100% regarding current recommendations. The possible use of incision drape was evaluated in 30 surgeries. It was used in 11 cases (36.7%). Iodine-impregnated incision drapes were used in 5 out of 11 cases (compliance=45.5%).

**Conclusion:** Compliance observations have proven to be a valuable instrument to assess the implementation of guideline compliance in OT. On this basis, we also created a questionnaire for general wards. Developing such instruments to evaluate prevention measures is a major part of the WACH-project and therefore it will contribute to improving prevention of SSI.

## 029-HYP

### Education of infection prevention in collaboration with physicians and nursing staff – how is the demand?

\*I. Hartlep<sup>1</sup>, I. F. Chaberny<sup>1</sup>, B. Schock<sup>1</sup>

<sup>1</sup>Leipzig University Hospital, Institute of Hygiene, Hospital Epidemiology and Environmental Medicine, Department of Diagnostics, Leipzig, Germany

The curriculum of hygiene is in constant change and so is the education of health-care professionals. An inter-professional collaboration at eye level between aspiring physicians and nursing staff is essential. Therefore, a hygiene-workshop was developed in collaboration with physicians and nursing staff, which was implemented in the curriculum of second semester students at the local medical vocational school. The goal of this workshop is to impart knowledge and practical skills to improve the understanding of development and prevention of nosocomial infections. Using a uniquely designed questionnaire, a survey (n=261) was conducted one week in advance of the workshop.

Questions about the student's state of knowledge and their possible need for certain topics (e.g. "I'd like to attend courses that are being supervised by nursing staff and physicians.") were included. Furthermore, students were asked about the appreciation of guideline-based hygiene behavior as well as the implementation of hygiene by physicians and nursing staff (Likert scale; 1= I disagree, 7= I agree). More interaction with nursing staff and physicians was demanded by most of the participants (M=6.02 vs. M=5.00; p<0.001). The appreciation of correct hygiene behavior was valued much higher among nursing staff compared to physicians (M=5.02 vs. M=4.29; p<0.001). Moreover, nursing staff reported a significantly higher self-observed compliance in implementing guideline-based hygiene compared to physicians (M=3.78 vs. 4.49; p<0.001). These findings show that an inter-professional collaboration between nursing staff und physicians as part of their education about infection prevention is highly needed. In addition to improving education, inter-professional exchange of knowledge at eye level should be implemented in order to create new perspectives for future healthcare professionals. These procedures may lead to better communication, mutual appreciation and improved cooperation between different groups of healthcare professionals and may also contribute to a higher compliance of guideline-based hygiene. As a result, the efficacy of infection prevention and patient care may benefit significantly.

## 030-HYP

### Nasal MRSA colonization of veterinary staff in German equine clinics

\*C. Cuny<sup>1</sup>, W. Witte<sup>1</sup>, U. Pansegrau<sup>2</sup>, W. Scheidemann<sup>3</sup>

<sup>1</sup>Robert Koch Institute, Section Nosocomial Infections and Antibiotic Resistance, Wernigerode, Germany

<sup>2</sup>Animal Hospital Lüsche, Bakum, Germany

<sup>3</sup>Veterinary Competence Centre Karthaus, Dülmen, Germany

Nosocomial infections with MRSA in equine clinics are common in many countries and associated with particular "hospital strains". Actually the veterinary clinic associated subpopulation of livestock associated MRSA CC398 represents the majority of the MRSA isolates.

When emerging in equine clinics these MRSA are also found as nasal colonizers of veterinary staff. Nasal MRSA carriage bears the risk for infections in case of predisposing conditions (e.g. wounds).

Here we report a study on nasal MRSA colonization of 325 staff members in 16 German equine clinics, on the frequencies at which barrier precautions were implemented, on conditions predisposing to MRSA carriage, and on results from typing the isolates.

The average prevalence of MRSA colonization was 17,5%. Wearing gloves when carrying out relevant activities was reported by 44% of the 143 veterinarians and by 33% of the other 182 staff members. Among the veterinary assistants MRSA carriage was less frequent when wearing gloves (8,3% vs. 22%, p0,044). Face masks were used by 8% of the veterinarians and 12,5% of veterinary assistants. Antibiotic consumption in the time period of 6 month prior to sampling proved to be a risk factor (p0,001) for MRSA colonization.

Among the 125 MRSA isolates 91% represented the veterinary hospital associated subpopulation of clonal complex CC398.

The prevalence of nasal MRSA colonization of veterinary staff is still high and above that observed for staff in other veterinary disciplines besides that attending conventional livestock. Results from typing indicate that acquisition seems to be mainly associated with work in the clinics.

Besides measures targeting dissemination of MRSA in equine clinics the implementation of barrier precautions for prevention of nasal colonization of hospital staff is urgently needed.

Key words: MRSA, horse clinics, colonization of staff

### 031-DKMP

Evaluation of Medicinal Plants Extract Against Biofilm Formation in *Pseudomonas Aeruginosa*

\*K. Nwanekwu<sup>1</sup>

<sup>1</sup>Imo State University, Microbiology, Owerri, Nigeria

**Method:** The antibacterial and biofilm inhibitory activity of leaf extracts of *Ocimum gratissimum*, *Moringa oleifera*, *Hibiscus sabdariffa*, *Azadirachta indica* and seed extract of *Garcinia kola* against clinical isolates of *Pseudomonas aeruginosa* were evaluated using the agar diffusion and microtiter assay methods.

**Results:** The ethanol extracts of *O. gratissimum* and *H. sabdariffa* exhibited significant antibacterial activity against the *P. aeruginosa* with diameter zones of inhibition of 25mm and 30mm with minimum inhibitory concentrations (MIC) of 0.16mg/ml and 0.18mg/ml respectively. While the ethanol extracts of *A. indica*, *M. oleifera* and *G. kola* showed no antibacterial effect against the test organism. However, all five plants extract inhibited the formation of biofilm with optical density (OD) values reduction from 0.168 to 0.160 for *O. gratissimum*, 0.170 to 0.151 for *H. sabdariffa*, 0.140 to 0.138 for *A. indica*, 0.145 to 0.137 for *M. oleifera* and 0.135 to 0.130 for *G. kola*. Out of five plants extract tested, *H. sabdariffa* exhibited the best growth and biofilm inhibition activity of *P. aeruginosa*.

**Conclusion:** The results of this study indicate that various concentrations of the plants extract particularly *H. sabdariffa*, may provide an alternative to control biofilm-related infections caused by *P. aeruginosa*.

### 032-DKMP

Value of the CXCL13 ELISA and the ReaScan® Lateral Flow Immunoassay in the Diagnosis of Lyme Neuroborreliosis.

\*K. Ziegler<sup>1</sup>, A. Rath<sup>2</sup>, C. Schoerner<sup>2</sup>, R. Meyer<sup>2</sup>, T. Bertsch<sup>3</sup>, F. Erbguth<sup>4</sup>, J. Steinmann<sup>1</sup>, J. Held<sup>2</sup>

<sup>1</sup>Klinikum Nürnberg, Institut für Klinikhygiene, Medizinische Mikrobiologie und Klinische Infektiologie, Nürnberg, Germany

<sup>2</sup>Universitätsklinikum Erlangen und Friedrich-Alexander-Universität

Erlangen-Nürnberg, Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Erlangen, Germany

<sup>3</sup>Klinikum Nürnberg, Institute of Clinical Chemistry, Laboratory Medicine and Transfusion Medicine, Nürnberg, Germany

<sup>4</sup>Klinikum Nürnberg, Klinik für Neurologie, Nürnberg, Germany

**Background and Objectives.** Diagnosis of Lyme neuroborreliosis (LNB) can be challenging in an early disease stage especially if *Borrelia*-specific intrathecal antibodies are still negative. This study aimed to assess the performance of CXCL13 ELISA and the ReaScan® CXCL13 lateral flow immunoassay (LFA) in the diagnosis of Lyme neuroborreliosis.

**Material and Methods:** In this dual-center case-control study 90 CSF samples were retrospectively analysed by the Euroimmun CXCL13 ELISA and the ReaScan® CXCL13 LFA. Overall, 34 CSF samples from patients with definite LNB, 10 samples from patients with possible LNB and 46 samples from patients with other predominantly inflammatory CNS diseases (non-LNB control group) were included. Patients with definite or possible LNB were classified according to the EFNS guidelines.

**Results.** CXCL13 ELISA was significantly elevated in all 34 patients with definite LNB (median 1409 pg/mL) compared to 46 control patients (median 20.7 pg/mL,  $p < 0.0001$ ). In the control group patients with possible LNB were not included. For a cut-off of 78.6 pg/mL a sensitivity of 100% and a specificity of 84.8 % (AUC 0.93) was calculated. The ReaScan® CXCL13 LFA was significantly elevated in 31 patients with definite LNB (median 223.5 arbitrary values) compared to 46 control patients (median 0 arbitrary units,  $p < 0.0001$ ). A cut-off of 22.5 arbitrary values (AV) had a sensitivity of 91.2 % and a specificity of 93.5 % (AUC 0.94). Overall, the agreement of the LFA with the ELISA was 90%; 98% for ELISA values  $< 250$  pg/mL; 22 % for ELISA values from 250-500 pg/mL and 96 % for ELISA values  $> 500$  pg/mL, respectively. The correlation between the CXCL13 ELISA and the LFA was  $r = 0.89$  and  $p < 0.0001$ .

**Conclusion.** CXCL13 ELISA and the ReaScan® CXCL13 LFA in CSF are reliable diagnostic tools for the identification of patients with definite LNB.

### 033-DKMP

Genomic diversity of *Achromobacter xylosoxidans* during the persistence in the airways of cystic fibrosis patients

\*S. Kampmeier<sup>1</sup>, A. Mellmann<sup>1</sup>, B. C. Kahl<sup>2</sup>

<sup>1</sup>University Hospital Münster, Institute of Hygiene, Münster, Germany

<sup>2</sup>University Hospital Münster, Institute of Medical Microbiology, Münster, Germany

### Introduction

*Achromobacter xylosoxidans* are increasingly recognized as emerging pathogens in patients suffering from cystic fibrosis (CF). Detection of these pathogens in the airways of CF patients is accompanied with an impaired pulmonary function.

### Objectives

To detect genomic clusters of *A. xylosoxidans* strains in different CF patients and to elucidate genetic alterations of these pathogens over time.

### Materials & methods

*A. xylosoxidans* isolated during 2006 to 2018 from sputa of CF patients were analyzed by whole genome sequencing using the Illumina NextSeq 500 platform. Using SeqSphere+ software version 6.0.0, all coding regions were compared in a gene-by-gene approach (core genome multilocus sequence typing, cgMLST) using *A. xylosoxidans* NCTC 10807 (GenBank accession number NZ\_LN831029.1) as a reference sequence. Genetic relation was displayed using the minimum-spanning tree algorithm, whereby close genetic relationship was assumed at 20 allele difference or less.

### Results

In total, 131 *A. xylosoxidans* strains isolated from 24 patients were analyzed by whole genome sequencing resulting in 13 clusters of two to 14 close related genotypes, whereby one cluster harbors genotypes isolated from three different patients, indicating a possible transmission of this pathogen. All other clusters only harbor isolates derived from one and the same patient. In three patients, isolates show a cluster of distantly related (21-350 alleles difference) genotypes. Ten isolates derived from one patient over time resulted in two different clusters of unrelated and distantly related genotypes.

## Conclusion

In the majority of cases one *A. xylosoxidans* clone persist in the airway of CF patients and alters genetically over time. Transmissions of *A. xylosoxidans* from one patient to another are possible, making infections control approaches more important.

## 036-DKMP

Inhibition of Glutaminyl cyclase of *Porphyromonas gingivalis* by small molecules - a promising new approach for the prevention and treatment of periodontitis

\*N. Taudte<sup>1</sup>, J. U. Rahfeld<sup>1</sup>, S. Eick<sup>2</sup>, D. Ramsbeck<sup>1</sup>, M. Linnert<sup>1</sup>, S. Schilling<sup>1</sup>, H. U. Demuth<sup>1</sup>, M. Buchholz<sup>1,3</sup>

<sup>1</sup>Fraunhofer Institute for Cell Therapy and Immunology, Drug Design and Target Validation, Halle (Saale), Germany

<sup>2</sup>University of Bern, Bern, Switzerland

<sup>3</sup>PerioTrap Pharmaceuticals GmbH, Halle (Saale), Germany

Chronical periodontitis is one of the most infectious inflammatory diseases worldwide, while *Porphyromonas gingivalis* as "keystone" pathogen and other bacteria as part of the "red complex" such as *Tannerella forsythia* play a crucial role in the development and progress of the disease. Currently periodontitis is treated only by non-surgical therapy as debridement and the application of either systemic or locally antibiotics or antimicrobial drug. Therefore an alternative therapy would be desirable which could be achieved by targeting of a physiological relevant bacterial enzyme of *P. gingivalis* for example through selective inhibition by a small molecule.

We and other authors identified an enzyme, glutaminyl cyclase (PgQC), which is essential for the virulence and fitness of the oral pathogen *P. gingivalis* and presumably for further oral pathogens like *Tannerella forsythia* and *Prevotella intermedia*.

Using a PgQC structure based approach (manuscript in preparation); first tool compounds were developed with compound activities in the nM range. We further applied the "Trojan horse strategy" by conjugating the QC inhibiting substructure with deuteroporphyrin. This conjugate binds specific the Haemophore-like protein (HusA) of the outer membrane of bacteria belonging to the phylum *Bacteroidetes* including the oral pathogens *P. gingivalis*, *T. forsythia* and *P. intermedia*. Thus, we ensure the uptake of our compound with high selectivity.

With this first lead compound the growth of *P. gingivalis*, *T. forsythia* and *P. intermedia* could be abolished at 0.5-1 µM, whereas the growth of *Aggregatibacter actinomycetemcomitans*, an example of a "non QC-expressing oral pathogen" or *Streptococcus gordonii*, an example of a "non QC-expressing naturally occurring bacteria", was rarely to none affected.

In conclusion, a compound was developed that inhibits specifically the growth of oral pathogens *P. gingivalis*, *T. forsythia* and *P. intermedia* by targeting a physiological essential enzyme. This could be a starting point for further development of an alternative treatment for periodontitis and saving broad spectra antibiotics for other clinical indications.

## 037-DKMP

Diagnosis and drug susceptibility of *Mycobacterium tuberculosis* from pulmonary specimens at Pasteur Institute of Algeria: Comparative study between classic Lowenstein-Jensen culture and BACTEC MGIT 960 system.

\*F. DJOUDI<sup>1</sup>, D. BENREMILA<sup>1</sup>, D. YALA<sup>2</sup>

<sup>1</sup>University Of Bejaia, Microbiology, Bejaia, Algeria

<sup>2</sup>Pasteur Institute of Algeria, National Reference Laboratory of Tuberculosis and Mycobacteria, Alger, Algeria

**Background:** tuberculosis is an old infectious disease and the causative agent is *Mycobacterium tuberculosis* complex. The direct diagnosis stills long and fastidious since microscopic examination, with Ziehl-Neelsen (Z-N) staining, even fast, lacks sensitivity. The culture on Löwenstein-Jensen (L-J), a reference method, sometimes takes up to ten weeks to obtain the result. In order to compensate the slow growth of cultures on solid media, new automated methods have been developed, including BACTEC MGIT 960, VERSA TREK, MBREDOX, BACTEC 460, allowing early diagnosis and drugs susceptibility testing, in addition to their good sensitivity and specificity.

**Materials and Methods:** The aim of this study is to verify the contribution of BACTEC MGIT 960 in the diagnosis of pulmonary tuberculosis, compared to microscopic examination and culture on L-J medium, at the tuberculosis and mycobacteria unite in Pasteur Institute of Algeria. Nine hundred and fourteen specimens were collected between January 2016 and April 2017. One hundred and seventy nine reported positive L-J culture and/or BACTEC MGIT 960.

**Results and Discuss:** Among the 179 cases, 155 were detected by the BACTEC MGIT 960, and confirmed by Ziehl control, L-J subculture and MPT64 immuno-chromatographic assay. On classic culture and Z-N staining, nevertheless, only 123 and 95 specimens respectively were positive. These results confirm the high susceptibility of BACTEC MGIT 960 in improving the diagnosis of tuberculosis in bacilli-poor specimens, compared to classic culture (p=0.037) and direct examination (p=0.014). Contamination rate was higher in L-J culture: 81/914 (8.86%), including 7 microscopic examination positive cases, whereas, in BACTEC MGIT 960, only 29/914 (3.17%) specimens were contaminated, with no positive microscopic examination cases.

**Conclusions:** The main advantage of BACTEC MGIT 960 is its ability to shorten the time of growth to an average of 7 days, compared to the solid medium. Nevertheless, there is an incompressible risk of contamination. Bacilloscopy and L-J culture remain complementary to this automat for a reliable diagnosis.

## 038-DKMP

Proof of Concept Evaluation of a New Diagnostic Device for Culture-Independent Microbiological Diagnosis of Infective Endocarditis

\*M. Karrasch<sup>1</sup>, W. Pfister<sup>2</sup>, M. Diab<sup>3</sup>, T. Doenst<sup>3</sup>, B. Löffler<sup>2</sup>, J. Rödel<sup>2</sup>

<sup>1</sup>Jena University Hospital, Clinical Chemistry and Laboratory Medicine, Jena, Germany

**Question:** Accurate and fast microbiological diagnosis of infective endocarditis (IE) is of vital importance for patient outcome.

**Methods:** Forty culture-negative heart valves were evaluated with a new multiplex-PCR cartridge (Unyvero™, Curetis AG, Holzgerlingen, Germany), advertised to detect several Gram-positive/-negative bacteria and fungi, together with several antibiotic resistance genes. Those POCT results were compared to conventional 16S rDNA PCR/sequencing results.

**Results:** Multiplex-PCR was positive in 13 cases [*Staphylococcus aureus* (n=5), *Enterococcus spp.* / *E. faecalis* (n=5), *ConS* (n=1), *Granulicatella adjacens* (n=1), *Abiotrophia adjacens* (n=1)]. Antibiotic resistances were found in 4 specimens, from which 2 specimens were without any pathogen identification. 16S rDNA PCR was positive in 20 cases. Consecutive sequencing identified those as *Staphylococcus spp* (n=6), *Enterococcus faecalis* (n=4), *Streptococcus spp.* (n=4), *Leifsonia shinshuensis* (n=1), *Granulicatella elegans* / *G. adjacens* (n=2), *Abiotrophia adjacens* (n=1). One case was positive in 16S PCR without any reliable signal in sequencing. When comparing both methods, identification was consistent in 9 cases and divergent in other 9 cases.

**Discussion:** This cartridge is easy to integrate into the daily microbiology laboratory workflow, and is less laborious than 16S sequencing PCR. For the application in routine IE diagnosis, the system needs to be optimized to include targets for viridans streptococci and HACEK group. In addition, problems with invalid resistance and pathogen target detection need to be fixed by the producer.

**Conclusion:** The analyzed system might be a future diagnostic tool for IE detection following assay optimization. In addition, a performance comparison with other similar culture-independent methods should be performed before using this system in routine diagnosis.

---

## 039-DKMP

### Nosocomial parainfluenza virus type 3 infections at a large German University Hospital between 2012 and 2017

\*M. Hönemann<sup>1</sup>, D. Martin<sup>1</sup>, U. G. Liebert<sup>1</sup>

<sup>1</sup>Institute of Virology, Leipzig University, Leipzig, Germany

**Question:** The clinical impact of parainfluenza virus infections is rarely studied systematically. The disease burden of parainfluenzavirus type 3 (PIV-3), however, is comparatively high and severe disease courses can occur in young children and immunocompromised patients. The aim of the study was to determine the seasonal dynamics, rate and clinical characteristics of nosocomial infections with PIV-3 in comparison to influenza viruses in five consecutive seasons.

**Methods:** A retrospective observational study was conducted at Leipzig university hospital (UKL) on patients with laboratory-confirmed infection with either PIV-3 or influenza from 1st October 2012 to 30th September 2017.

**Results:** A total of 309 patients were tested positive for PIV-3 and 638 patients for influenza virus. Nosocomial infection

occurred in 13.9% (n = 89) of all influenza cases. Rates of nosocomial infection differed between influenza virus types and seasons with up to 17.4 % for influenza A/H3N2 and up to 22.2 % for season 2016/17, respectively. Regarding PIV-3, 23.9% (n = 74) of the cases were nosocomial and rates did not vary significantly between the seasons. For both, PIV-3 and influenza virus, community acquired and nosocomial cases differed in underlying medical conditions and regarding the role of immunosuppression.

**Conclusions:** Nosocomial infections account for a substantial proportion of infections with parainfluenzavirus type 3, especially in immunocompromised patients. Knowledge of the baseline rate of nosocomial viral infections could contribute to the implementation of appropriate infection control measures.

---

## 041-DKMP

### Metabolic insights into the clinical urethral catheter isolate *Myroides spp.* A21

\*D. Hiller<sup>1</sup>, S. Barthels<sup>1</sup>, M. Jahn<sup>1</sup>, D. Jahn<sup>1</sup>

<sup>1</sup>TU Braunschweig, Institute of Microbiology, Braunschweig, Germany

**Introduction:** The opportunistic pathogen *Myroides spp.* emits unique volatile organic compounds (mVOCs) which act as infochemicals with in part detrimental effects on other microorganisms during infection. The biochemical reaction pathways of volatiles and their role as virulence factors has to be elucidated in more detail.

**Objectives:** We try to shed light on the virulence of one clinical catheter isolate (A21) and the role of mVOCs from *Myroides spp.* during infection process of human cells.

**Material & methods:** We used BIOLOG Phenotype MicroArrays™, double immunofluorescence microscopy, OLSA, GC/MS, Co-culture, KEGG pathway generation, Omics (metabolomic, transcriptomic, proteomic, volatilomic), amino-acid analytic with HPLC-FLD.

**Results:** Preliminary results revealed that co-culture experiments of a T24 cells, a bladder carcinoma cellline, with the *Myroides* A21 isolate revealed an invasive character. *Proteus mirabilis* loses its swarming capacity and *Pseudomonas aeruginosa* its green colouring when incubated with the A21 mVOCs. Current BIOLOG arrays provided information about different used carbon and nitrogen sources to let us establish a metabolomic map about *Myroides*.

The clinical catheter isolate A21 is able to metabolize amino-acids as the sole energy source. The newly developed defined minimal medium (Artificial *Myroides* Medium, AMM) was essential for further metabolite and volatile analyses. Time resolved amino-acid HPLC-FLD analyses of *Myroides* supernatants in AMM with glutamin, asparagine, cysteine and tryptophane resulted in consumption of more amino-acids than in AMM without additional amino-acids.

Co-culture experiments of T24 cells with *Myroides* showed interesting GC/MS data of intracellular metabolites. On the one hand *Myroides* was set in direct contact with the host and the other hand *Myroides* was indirectly cultivated with the host in two different chambers divided by a septum. The intracellular metabolites like citrate, aspartate, malate and 2-ketoglutarate are strongly reduced in comparison to the medium. Fumaric acid was the only TCA-cycle metabolite

which was not reduced during co-cultivation (direct contact) in contrast to indirect cultivation

#### 042-DKMP

##### **Five-Year Surveillance of the Prevalence of *C. trachomatis* serovars L1-L3 (Lymphogranuloma venereum) in a High-Risk Patients' Cohort**

\*D. Schmidt<sup>1</sup>, U. Scharmann<sup>1</sup>, S. Esser<sup>2</sup>, J. Buer<sup>1</sup>, P. M. Rath<sup>1</sup>

<sup>1</sup>University Hospital Essen, Institute of Medical Microbiology, Essen, Germany

<sup>2</sup>University Hospital Essen, Clinic of Dermatology, Essen, Germany

**Introduction:** Lymphogranuloma venereum (LGV), caused by *Chlamydia trachomatis* serovars L1-L3, is a rare sexually transmitted disease. Originally a tropic disease, LGV has spread across Europe since 2003, mainly affecting men who have sex with men (MSM). The disease begins with a primary lesion in the anal region and develops rapidly into severe proctitis and proctocolitis. Eradication requires prolonged antibiotic treatment and often surgical intervention, too. Data from Germany are scarce due to the abolishment of compulsory notification of LGV in 2001. Additionally, only a few laboratories have implemented molecular tests for the detection of *C. trachomatis* serovars L1-L3 despite the recent availability of commercial tests. **Material/methods:** Between 2014 and 2019 we have analyzed 2265 urogenital and anal samples from 882 male patients (age 16-80 years, median = 38 years) who reported periodically at the HIV polyclinic of the University Hospital Essen. All samples were tested for the presence of *C. trachomatis* using commercial qPCR assays. Samples tested positive for *C. trachomatis* were retested using an inhouse qPCR detecting *C. trachomatis* serovars L1-L3 only. To further manifest our qPCR results for serovars L1-L3 clinical manifestations were also taken into account. **Results:** 218 samples (9.6%) from 198 patients were tested positive for the presence of *C. trachomatis*. 38 samples (17.4%) from 34 patients were tested positive for the presence of *C. trachomatis* serovars L1-L3. 16 (47%) of these patients had clinical manifestations of proctitis. For 18 of these patients clinical manifestations of *Chlamydia* related proctitis had not been expressly mentioned in their electronic health report. **Conclusions:** Screening for *C. trachomatis* in general and for serovars L1-L3 in particular is recommended for a high-risk patients' cohort. Despite an increase of samples from 237 in 2014 to 893 samples in 2019 this did not result in a higher positive rate for *C. trachomatis* and/or serovars L1-L3. Reason for this may be found in a greater awareness of the problem and a good surveillance of this particular patients' cohort at our clinic.

#### 043-DKMP

Whole genome analysis of one XDR *Mycobacterium tuberculosis* isolate, from Algeria.

\*D. BENREMILA<sup>1</sup>, F. DJOUDI<sup>1</sup>, S. BATTAGLIA<sup>2</sup>, E. BORRONI<sup>2</sup>, A. CABIBBE<sup>2</sup>, D. M. CIRILLO<sup>2</sup>

<sup>1</sup>University of Bejaia, Algeria, Department of Microbiology, , Bejaia, Algeria

<sup>2</sup>IRCCS San Raffaele Scientific Institute, TB Supranational Reference Laboratory,, Milan, Italy

**Background:** The anti-tuberculosis sensitivity testing remains essential for a complete diagnosis and provides better medical management of the patient. However, conventional methods remain complex and time consuming, while automated methods don't identify all genetic loci associated with TB drug resistance. Whole genome sequencing (WGS) addresses this problem and allows reliable prediction of drug susceptibility phenotype within a short time with exhaustive results for better prescribing.

**Material and Methods:** In this study, we performed a WGS of the first Extremely Drug Resistant isolate of *M. tuberculosis* (XDR-TB), described in our works on tuberculosis. *M. tuberculosis* isolate was identified by direct examination, according to Ziehl-Neelsen (Z-N) staining and bacterial culture on Lowenstein-Jensen (L-J) medium. Identification was confirmed by whole genome sequencing. Genomic DNA was extracted from 1 ml of MGIT culture, using a lysis buffer and protease K. Total DNA was using Promega Maxwell 16 cell DNA purification KIT (AS 1020). A library preparation was performed by using the Nextera XT DNA Library prep kit. All gDNA samples were fragmented, with the nextera XT transposome, and adapters sequences added to the both ends of each DNA fragment generated, then two index primers were added for all samples. Tagmented DNA was amplified with PCR. DNA was sequenced on illumina next seq500 system. Interpretation of the fastQ files generated was done by PHYRESSE analysis.

**Results:** The most frequent polymorphism detected in the *katG* gene was Ser315Thr. In *rpoB*, the Ser450Leu amino acid change. Mutation in *embB* gene with amino acid change of Met-Val at position 306 was also reported. Mutations were also reported in *gid*, *rrs* and *rpsL*. In *pncA* gene, conferring pyrazinamide resistance, Ala146Pro was identified. For Fluoroquinolones, Asp94Asn mutation in *gyrA* gene identified. Many other mutations were detected.

**Conclusion:** Resistance to many drugs, never used in Algeria, were detected by WGS.

#### 044-DKMP

##### **Multivalent neo-glycoconjugates as binders and scavengers of bacterial enterotoxins**

V. Heine<sup>1</sup>, R. R. Rosencrantz<sup>1,2</sup>, C. Dey<sup>1</sup>, A. Jans<sup>3</sup>, S. Boesveld<sup>4</sup>, A. D. Mandić<sup>4</sup>, N. Anwar<sup>3</sup>, H. Pelantová<sup>5</sup>, V. Křen<sup>5</sup>, U. Schnakenberg<sup>6</sup>, M. Moeller<sup>3</sup>, C. Trautwein<sup>4</sup>, G. Selge<sup>4</sup>, P. Strnad<sup>4</sup>, A. J. C. Kuehne<sup>3</sup>, \*L. Elling<sup>1</sup>

<sup>1</sup>RWTH Aachen University, Laboratory for Biomaterials, Institute for Biotechnology and Helmholtz-Institute for Biomedical Engineering, Aachen, Germany

<sup>2</sup>Fraunhofer-Institute for Applied Polymer Research IAP, Potsdam, Germany

<sup>3</sup>RWTH Aachen University, DWI – Leibniz Institute for Interactive Materials, Aachen, Germany

<sup>4</sup>University Hospital, RWTH Aachen University, Department of Internal Medicine III, Aachen, Germany

<sup>5</sup>Czech Academy of Sciences, Institute of Microbiology, Prague, Czech Republic

<sup>6</sup>RWTH Aachen University, Institute of Materials in Electrical Engineering 1, Aachen, Germany

**Introduction:** Bacterial enterotoxins cause intestinal diseases with severe outcome. Increasing antibiotic resistance of enterotoxin secreting bacteria triggers the necessity for new therapies. The initial step of infection is binding of enterotoxins to cell-surface glycans of intestinal epithelial cells. Binding and scavenging of enterotoxins by neo-glycoconjugates could inhibit disease progression.

**Objectives:** Multivalent presentation of selective glycan ligands are crucial for binding and scavenging of enterotoxins. Together with our cooperation partners, we developed glycopolymers, neo-glycoproteins, and glycan-functionalized microgels for binding and scavenging of toxin A (TcdA) from *Clostridium difficile* and cholera toxin (CT) from *Vibrio cholera*.

**Material & methods:** Glycopolymer brushes were synthesized on a gold sensor surface and modified to obtain the Galili epitope, a non-human glycan, known for binding the TcdA-receptor domain.<sup>[1]</sup> A multivalent BSA neo-

glycoprotein glycan ligand library presenting 40 different glycans was screened for TcdA and CT binding.<sup>[2]</sup> Microgels functionalized with the glycan epitope GM1a were synthesized and investigated for CT binding and scavenging.<sup>[3]</sup>

**Results:** Localized surface-plasmon resonance reveals nanomolar binding of TcdA-R to a Galili-functionalized glycopolymer brush biosensor.<sup>[1]</sup> BSA neo-glycoprotein with Lewis<sup>y</sup>-Lewis<sup>x</sup> glycans facilitates scavenging of TcdA and protects human colon cells in the presence of TcdA.<sup>[2]</sup> GM1a functionalized microgels scavenges CT and protect colorectal cells.<sup>[3]</sup>

**Conclusion:** Multivalent neo-glycoconjugates are highly effective in binding the bacterial enterotoxins TcdA and CT.

**Funding:** DFG - SFB 985 project C3.

[1] R. R. Rosencrantz, V. H. Nguyen, H. Park, C. Schulte, A. Böker, U. Schnakenberg, L. Elling, *Anal. Bioanal. Chem.* **2016**, *408*, 5633-5640.

[2] V. Heine, S. Boesveld, H. Pelantová, V. Křen, C. Trautwein, P. Strnad, L. Elling, *Bioconj. Chem.* **2019**, doi: 10.1021/acs.bioconjchem.9b00486.

[3] S. Boesveld, A. Jans, D. Rommel, M. Bartneck, M. Moller, L. Elling, C. Trautwein, P. Strnad, A. J. C. Kuehne, *ACS Appl. Mater. Interfaces* **2019**, *11*, 25017-25023.

---

#### 045-DKMP

The author has not agreed to a publication.

---

#### 046-DKMP

Typing method for identifying and discriminating salivarius group streptococci using dephospho-coA kinase (*coaE*) as target gene

\*M. M. H. Abdelbary<sup>1</sup>, G. Wilms<sup>1</sup>, G. Conrads<sup>1</sup>

<sup>1</sup>RWTH Aachen University Hospital, Division of Oral Microbiology and Immunology, Department of Operative and Preventive Dentistry and Periodontology, Aachen, Germany

**Introduction:** Viridans group streptococci are commensals but also important human pathogens such as *S. pneumoniae*. Identification and molecular typing of viridans group streptococci are challenging, especially for members of the salivarius group.

**Objectives:** We developed a single-locus typing method that is able to identify the highly phylogenetically related members of the salivarius group (*S. salivarius*, *S. vestibularis* and *S. thermophilus*). This typing approach is based on the amplification and sequence analysis of the housekeeping gene dephospho-coA kinase (*coaE*).

**Materials & methods:** We first analysed the *coaE* gene sequences of 118 publicly available genomes representing 20 different bacterial species (16 streptococcal and 4 non-streptococcal species). We designed the primers flanking a variable region of the *coaE* gene of *S. salivarius* strain NCTC 8618 (ATCC 7073) as reference genome. In addition, a total of 32 salivarius group streptococci isolates that were collected from various clinical backgrounds were characterized using the newly developed *coaE* typing approach.

**Results:** The *coaE*-based phylogeny distinguished between streptococcal and non-streptococcal genomes and *coaE* gene sequences were species-specific. The maximum likelihood tree based on *coaE* gene sequences alignment (594 bp) of all investigated salivarius group genomes revealed two main clades representing *S. salivarius* and *S. thermophilus* species-specifically. In contrast, *S. vestibularis* had several phylogenetically distinct clusters located between the *S. salivarius* and *S. thermophilus* clades.

**Conclusion:** The developed *coaE* typing approach was able to precisely identify the phylogenetically very closely related members of salivarius group streptococci. Our results emphasised a sister-like phylogenetic relationship between *S. vestibularis* and *S. thermophilus* and – supported by literature – that they both have evolved from the common ancestor *S. salivarius*. We therefore conclude, that using the *coaE* typing approach is a fast and cost-effective first line typing tool for salivarius group streptococci and will overcome some disadvantages of other typing methods.

---

#### 047-DKMP

##### Identification of genetic factors increasing carbapenem resistance in *Klebsiella pneumoniae* with *bla*<sub>OXA-48</sub>

\*M. Cremanns<sup>1</sup>, S. G. Gatermann<sup>1</sup>, N. Pfennigwerth<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Department of Medical Microbiology, Bochum, Germany

**Introduction:** Carbapenemase-producing Enterobacterales became the most clinically significant multi-drug resistant bacteria. In Germany, *Klebsiella pneumoniae* with *bla*<sub>OXA-48</sub> are most frequently detected, as shown in the annual report 2018 of the National Reference Laboratory for multidrug-resistant gram-negative bacteria (NRZ). Although OXA-48 usually mediates distinct resistance to carbapenems some clinical isolates show low minimal inhibitory concentrations (MIC) of carbapenems and are categorized susceptible using EUCAST breakpoints.

**Aim:** The aim of this study is to reveal possible genetic causations for varying MICs of carbapenems in *K. pneumoniae* with *bla*<sub>OXA-48</sub>.

**Material & Methods:** Clinical *K. pneumoniae* isolates with *bla*<sub>OXA-48</sub> showing low MICs of carbapenems were put under gradually rising selective pressure of meropenem to select for mutants with elevated MICs. This was done with disc diffusion or with broth macrodilution. Whole genome sequencing were performed with the low MIC primary isolates and the high MIC selected mutants to check for mutations. A hybrid assembly of Illumina MiSeq and Oxford Nanopore sequencing data was carried out to generate whole genome sequences.

**Results:** Only few spontaneous mutations were found comparing genome sequences of primary isolates and corresponding mutants. Mutations or IS elements possibly leading to elevated MICs were predominantly found in genes associated with outer membrane porins. To check if these genomic alterations lead to elevated MICs of carbapenems, they were integrated into the primary clinical isolates via CRISPR/Cas system. Clinical *K. pneumoniae* isolates with *bla*<sub>OXA-48</sub> showing high MICs will be screened for the found mutations.

**Conclusion:** There are only few single mutations having huge impact on the MICs of carbapenems in *K. pneumoniae* with *bla*<sub>OXA-48</sub> as shown by sequencing data of clinical isolates and selected mutants. Clinical *K. pneumoniae* isolates with high MICs of carbapenems will be screened for the found or



similar mutations in the same genes to check if *bla*<sub>OXA-48</sub> or *bla*<sub>OXA-48</sub> in combination with an mutation in an appropriate gene leads to high MIC in clinical isolates.

## 048-DKMP

Novel monoclonal antibodies for detection and serotyping of *Legionella* spp.

\*A. Helming<sup>1</sup>, E. Müller<sup>2</sup>, R. Ehricht<sup>2</sup>, M. Fischer<sup>1</sup>

<sup>1</sup>fzmb GmbH, IVD, Erfurt, Germany

<sup>2</sup>Leibniz-IPHT, InfectoGnostics Research Campus, Jena, Germany

## Introduction

*Legionella* is increasingly recognized as the causative agent of pneumoniae. Most cases are caused by *L. pneumophila* serotype 1, but other serogroups and species like *L. anisa* may also be pathogenic. Since the widely used urinary antigen test detects only *L. pneumophila* serotype 1 and the gold standard of *Legionella* detection is still time consuming cultivation, there is a growing demand on fast and solid detection of all serogroups as well as non-pneumophila species.

*Legionellae* are often associated to amoeba or biofilms, resulting in modified surface structures, which limit the properties of existing antibody-based detection systems. Further, *Legionellae* may enter a VNBC-state, which limits cultivation.

## Objectives

In order to provide new tools for an antibody-based detection of all potential pathogenic *Legionellae* a novel panel of monoclonal antibodies was raised against *L. pneumophila* and non-pneumophila species.

## Material & Methods

Monoclonal antibodies were developed using hybridoma technique. Immunisations were carried out with antigen variants carrying specific surface structures due to different cultivation methods (e.g. amoeba-associated). Serotype-independent surface structures we also employed. Experiments were performed with 29 *Legionella* strains including 9 substrains of SG 1 and representatives for SG 2-14 as well as 7 non-pneumophila strains.

## Results

25 monoclonal antibodies were raised. Three single antibodies react with all serotypes of *L. pneumophila*. Other mABs recognize separate serotypes or more than one. Using mixtures of these mABs detection of all serogroups is feasible. One antibody turned out to detect most of the non-pneumophila species like *Legionella anisa*. Microarray studies revealed potential for SG-discrimination via specific microarray-patterns.

## Conclusion

The developed panel of monoclonal antibodies offers great opportunities for the establishment of novel assay platforms for a fast and reliable detection of all existing *Legionella pneumophila* serotypes and substrains, as well as relevant non-pneumophila species.

## 049-DKMP

Direct MALDI identification from positive blood cultures: evaluation of Rapid Sepsityper with a large collection of routine samples

\*M. Cordovana<sup>1</sup>, A. Zignoli<sup>1</sup>, S. Ambretti<sup>2</sup>

<sup>1</sup>University Hospital Policlinico Sant'Orsola-Malpighi of Bologna, Microbiology, Bologna, Italy

<sup>2</sup>University Hospital of Bologna Policlinico Sant'Orsola-Malpighi, Microbiology, Bologna, Italy

## Background

The rapid identification of the causative agent of sepsis plays a key role for the adoption of an appropriate antibiotic therapy, and hence is crucial for the patients' clinical outcome.

In this study, we evaluate the implementation into the routine workflow of the Rapid Sepsityper kit for the rapid bacterial identification by MALDI-TOF MS directly from positive blood culture bottles.

## Methods

From February 2018 to September 2019, a total of 5,047 routine positive blood cultures samples underwent direct MALDI identification by Rapid Sepsityper (Bruker Daltonik).

The bacterial pellet extracted from 1 ml of positive blood culture by the lysis/centrifugation Sepsityper protocol was spotted directly onto the MALDI target for the species identification.

For 1,648 samples, the same pellet was then used to set up the antibiotic susceptibility testing by Microscan WalkAway (Beckman Coulter) broth microdilution panels.

## Results

Overall, the Rapid Sepsityper enabled the direct identification of 4,303/4,848 (88.2%) monomicrobial samples. It showed a very good performance for enterobacteria, non-fermenting gram-negative rods, staphylococci and enterococci. The missed identification were restricted mainly to a few groups of microorganisms (streptococci, corynebacteria, *Bacteroides* spp. and yeasts).

Among the polymicrobial samples, in 62/200 samples both species were identified, in 73/200 only one species, in 65 sample none of them.

Susceptibility testing was successful for 1549/1648 samples (94%) (for 99 samples the growth in the panel was insufficient).

## Discussion

The Rapid Sepsityper proved to be a reliable and robust method for the bacterial identification directly from positive blood cultures, with an excellent efficacy (near to 100%) for the most clinically relevant causative agents of sepsis (enterobacteria, *S. aureus*, enterococci). It enables to deliver a result in a very short time (around 1 h for a batch of 10-15 samples).

Further, the same bacterial pellet used for the MALDI identification proved to be suitable to set up the antibiotic susceptibility testing, simplifying and speeding up the routine workflow and the time-to-report.

---

## 050-DKMP

Evaluation of MICRONAUT-S Anaerobes MIC broth microdilution panels for antibiotic susceptibility testing of anaerobes

\*M. Cordovana<sup>1</sup>, S. Ambretti<sup>2</sup>

<sup>1</sup>University Hospital Policlinico Sant'Orsola-Malpighi of Bologna, Microbiology, Bologna, Italy

<sup>2</sup>University Hospital of Bologna Policlinico Sant'Orsola-Malpighi, Microbiology, Bologna, Italy

**Background:** Susceptibility of anaerobes to antibiotics has become unpredictable during the last decades, and empirical treatment of anaerobic infections is no longer satisfactory. Accurate and routine-friendly methods for MIC determination of anaerobes are demanded. Here, we evaluate the performance of a commercially available microdilution panel in comparison to a gradient MIC Strip test.

**Methods:** N=300 anaerobes clinical isolates (83 species, 31 genera) were tested with the MICRONAUT-S Anaerobes MIC panel (MERLIN Diagnostika, Germany), comprising penicillin, ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, meropenem, imipenem, ertapenem, metronidazole, clindamycin, vancomycin, doxycycline, moxifloxacin and tigecycline. The same bacterial suspension was used for the panel inoculum, and for testing by MIC Strips (Liofilchem, Italy). The panels were incubated at 37 °C in anaerobic conditions for ≥24 h, and read visually. In case of no bacterial growth for the growth control, incubation was prolonged to 48-72 h. Results were interpreted according to EUCAST guidelines. Comparison was performed in terms of essential agreement, category agreement and error rates.

**Results:** Category agreement with MIC strips resulted overall >97% (from 93.9% to 99.7%). Essential agreement resulted 92.9% for piperacillin/tazobactam, and >96% for all the other antibiotics. Overall, n=53 minor errors (1.8%), n=4 major errors and n=24 very major errors were observed (8 of which resulting from a difference of ≤1 dilution steps, and 15 restricted to ertapenem). For most isolates (219/300) the panels were readable after overnight incubation, in 67 cases after 48 h, in 11 after 72 h, in 4 after 96 h (*Dialister* and *Porphyromonas*).

**Conclusions:** The MICRONAUT-S Anaerobes MIC panels proved to be a reliable broth microdilution method for antibiotic susceptibility testing of anaerobes, providing results consistent with gradient methodology, with both fast and slow growing species, in most cases in 18-24 hours. The ease-of-handling and -result interpretation makes this method suitable for implementation into routine workflows.

---

## 051-DKMP

Azithromycin possesses biofilm-inhibitory activity against *Klebsiella pneumoniae* and potentiates colistin to combat klebsiella-associated infection

\*O. Moshynets<sup>1</sup>, O. Krykunov<sup>2</sup>, O. Iungin<sup>1</sup>, R. Jerdan<sup>3</sup>, G. Filonenko<sup>4</sup>, A. Spiers<sup>3</sup>

<sup>1</sup>Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, Kiev, Ukraine

<sup>2</sup>Amosov National Institute of Cardiovascular Surgery, Kiev, Ukraine

<sup>3</sup>School of Applied Sciences, Abertay University, Dundee, United Kingdom

<sup>4</sup>Center for Pediatric Cardiology and Cardiac Surgery, Kiev, Ukraine

Gram-negative infections have become a global health problem with *Klebsiella pneumoniae* recognised as one of the most dangerous. An intrinsic capability of many pathogens is the ability to form a biofilm which underlies

persistent, survival, and resistance to the immune system and antibacterial therapy, with biofilms generally providing phenotypic resistance when genetic resistance might be restricted. However, this may not always be the case, as *P.aeruginosa* biofilms are susceptible to azithromycin (AZM) whereas planktonic cells are not. Like *P.aeruginosa*, *K.pneumoniae* also forms biofilms and shares similar antibiotic resistance profiles, and we have hypothesized that some macrolides might possess similar anti-biofilm activity against *K.pneumoniae*.

We aimed to screen all macrolides following their biofilm-suppression and colistin-synergy in *K. pneumoniae* culture.

We have evaluated the anti-biofilm and bactericidal effectiveness of eight macrolides against *K.pneumoniae* ATCC1003, seven hospital isolates and five multidrug resistant (MDR) hospital isolates, with AZM showing significant anti-biofilm effects. Although colistin, an antibiotic of choice for MDR *K.pneumoniae*, showed low effectiveness against both planktonic and biofilm cultures, we found the combination of colistin and AZM led to a synergistic effect, improving the Cmic of colistin up to serum Cmax levels which could increase the therapy effectiveness of colistin in treating *K.pneumoniae* infections. Based on these findings, we applied the combination therapy for a MDR *K.pneumoniae* severe infection associated with a bad prognosis. Specifically, there were two clinical cases where MDR *K.pneumoniae* sensitive only to colistin was identified and an antibiotic therapy of imipenem, colistin and azithromycin were applied. In both cases the infection completely eradicated in 4 weeks.

Our results suggest that combination therapeutic approach using anti-biofilm compounds such as AZM with a task-specific antibiotic can be used to combat infections caused by MDR *K.pneumoniae*, and that the use of AZM to potentiate colistin may prove an effective solution for PDR and MDR infections.

---

## 052-DKMP

Identification of potential factors interfering with molecular diagnostics for bloodstream infections

\*S. Huber<sup>1</sup>, J. Weinberger<sup>2</sup>, M. Pilecky<sup>3</sup>, A. Schildberger<sup>3</sup>, V. Weber<sup>3,4,5</sup>, D. Orth-Höller<sup>1</sup>, A. E. Posch<sup>2</sup>

<sup>1</sup>Medical University Innsbruck, Institute of Hygiene and Medical Microbiology, Innsbruck, Austria

<sup>2</sup>Ares Genetics GmbH, Vienna, Austria

<sup>3</sup>Danube University Krems, Center of Biomedical Technology, Department of Health Science and Biomedicine, Krems, Austria

<sup>4</sup>Danube University Krems, Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis, Department of Health Science and Biomedicine, Krems, Austria

<sup>5</sup>Danube University Krems, Department of Biomedical Research, Krems, Austria

## Introduction

Bloodstream infections (BSI) call for a fast and accurate identification of causative pathogens. Thus, in recent years scientists focused on the development of molecular diagnostic approaches to serve these needs. Despite several advantages compared to blood culture, these systems, in particular PCR-based approaches (eg. LightCycler SeptiFast (SF); Roche Diagnostics, Penzberg, Germany) still suffer from limitations like inhibition due to interfering matrix components.

## Objectives

Aim of this study was to identify potential factors interfering with molecular diagnostics of BSI directly from blood.

## Methods

In this retrospective study 2148 SF results performed at the Institute of Hygiene and Medical Microbiology, Medical University Innsbruck, Austria from January 2013 to April 2019 were analyzed regarding general, laboratory and medical parameters that might be associated with test inhibition by univariate analysis with Wilcoxon and Fisher exact test.

## Results

The overall inhibition rate in this study was 9.08% (n=195). Within general parameters the ward where the patient was hospitalized was significantly associated with inhibition (q-value <0.05) with the highest inhibition risk at the Department of Surgical Anesthesia (14.4%). For laboratory parameters cut off values for leukocyte counts of <5.700 cells/ $\mu$ L and  $\geq$ 26.700 cells/ $\mu$ L could be significantly associated with a low (5%) and high (67%) inhibition risk (q-value <1 $\times$ 10<sup>-9</sup>). Samples with a leukocyte count between this cut off values showed a significant increase of inhibition from 33% to 69% when hydrocortisone was applied during hospitalization (q-value <0.05).

## Conclusion

This study investigated inhibitory factors of SF, a molecular test for BSI diagnostics. We identified the leukocyte count as the main factor for inhibition. In case of a moderate to high leukocyte count (5.700 to <26.900 cells/ $\mu$ L) the additional application of hydrocortisone significantly increased the risk of inhibition. These results give valuable information for further development of molecular tests. Whether other commercially available tests suffer from the same problem has to be further investigated.

### 053-DKMP

**Cutibacterium acnes** as cause for endocarditis: How to differentiate between contamination and infection?

\*L. Kursawe<sup>1</sup>, J. Kikhney<sup>1</sup>, A. Wießner<sup>1</sup>, S. Eichinger<sup>2</sup>, W. Eichinger<sup>2</sup>, H. Grubitzsch<sup>3</sup>, A. Moter<sup>1</sup>

<sup>1</sup>Charité - Universitätsmedizin Berlin, Biofilmzentrum, Institut für Mikrobiologie und Infektionsimmunologie, Berlin, Germany

<sup>2</sup>Hospital Bogenhausen, Department of Cardiac Surgery, München, Germany

<sup>3</sup>Charité-Universitätsmedizin Berlin, Department of Cardiovascular Surgery, Berlin, Germany

**Introduction:** Timely diagnosis of Infective Endocarditis (IE) is crucial for patient outcome but may be challenging. In particular, when culture or PCR-techniques identify skin flora, like *Cutibacterium* (*Propionibacterium*) *acnes*, the relevance often remains questionable due to suspected contamination during sampling.

**Objective:** We aimed to detect *C. acnes* in heart valve tissues of IE patients by Fluorescence in situ hybridization (FISH). Thus, we aimed to discriminate between contamination and infection and to explore the source of *C. acnes* IE.

## Methods:

Using FISH with a specific *C. acnes* probe combined with 16S rRNA PCR and sequencing, we identified and visualized

*C. acnes* directly within explanted heart valve tissue sections. Skin samples from sternal wound edges were taken from two of these patients to evaluate *C. acnes* colonization at the incision sites as possible source of the pathogens.

## Results:

In 13 patients, FISH and PCR detected exclusively *C. acnes* as causative agent of IE. Blood cultures remained negative in 11 patients, and valve tissue culture was positive in 8 of these 13 cases. Moreover, FISH showed impressive biofilm formation and activity of *C. acnes* in 5 cases even after treatment with antibiotics. On the other hand, in one case, FISH and PCR identified *Streptococcus gallolyticus* biofilms on the heart valve instead of *C. acnes* that had been grown in culture.

Skin samples from two patients revealed high numbers of *C. acnes* in biofilms located at the infundibulum of chest hair follicles.

**Conclusion:** *C. acnes* can form impressive biofilms on heart valves. FISH is a valuable tool for proving or rejecting diagnosis of *C. acnes* endocarditis. Furthermore, FISH showed a high load of *C. acnes* on chest hair follicles, supporting the hypothesis of contamination of the heart valve during skin incision at surgery.

### 054-DKMP

**Water samples containing increased ferric ions significantly reduce recovery rates of coliform and *E. coli* on selective chromogenic media**

\*R. Rösner<sup>1,2</sup>, A. Bommer<sup>1,2</sup>, T. Kuczius<sup>1,2</sup>

<sup>1</sup>Institute of Hygiene, University Hospital Münster, Münster, Germany

<sup>2</sup>University of Münster, Münster, Germany

Drinking water as necessary commodity for survival of living beings is one of the best controlled foods. In order to achieve drinking water quality in accordance with the German drinking water ordinance (TrinkwV) no fecal indicator bacteria such as coliform and *Escherichia coli* are allowed to be present and thresholds of chemicals must not be exceeded. The microorganisms are detectable in water samples using the membrane filtration technique followed by incubation on selective chromogenic coliform agar (CCA) with blue and red colored colony forming units (cfu) representing *E. coli* and coliforms, respectively. Chromogenic agar such as ESB, OXA and CARBA plates were used for detection of antibiotic multi resistant *Enterobacteria* as well. During analyses we observed that water samples with increased ferric ion contents (threshold in drinking water is 0.2 mg/l) present lower recovery rates than samples with low iron concentrations. Consequently, decreased recovery may result in false negative results. This phenomenon was not seen when Colilert was used as alternative method without membrane filtration. To analyze the influence of ferric ions on the recovery rates we analyzed specifically the cfu of *E. coli*, *Klebsiella pneumoniae* and *Citrobacter freundii* which were admitted to water samples with four different added iron concentrations as well as real water samples with various iron contents. We found a reproducible reduction of the cfu of at least 60% when samples contain > 1 mg/l iron on selective plates while no reduction was observed on non-selective Luria broth. We suggest that high iron contents on filter membranes in combination with ingredients of chromogenic agars may have an important influence on the recovery rate and analyze various components of the media in detail.

---

## 055-DKMP

### Correlation between predicted antimicrobial resistance genes and resistance phenotype in German *Campylobacter jejuni* clinical isolates

\*S. Banerji<sup>1</sup>, P. Auraß<sup>1</sup>, A. Tille<sup>2</sup>, A. Fruth<sup>1</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>Robert Koch-Institute, FG11, Wernigerode, Germany

<sup>2</sup>Robert Koch-Institute, Wernigerode, Germany

**Introduction:** Campylobacteriosis is the most frequently reported food-borne bacterial infection in Europe. *Campylobacter jejuni* accounts for the majority of reported infections. Here we investigate the correlation between predicted AMR genes and the AMR phenotype in clinical isolates.

**Objectives:** Assessment of genotypic and phenotypic correlation of antimicrobial susceptibility in German clinical *Campylobacter* isolates

**Materials & methods:** Whole genome sequencing was performed with an Illumina MiSeq v3. *In silico* AMR prediction was performed with Ariba (<https://github.com/sanger-pathogens/ariba>) and kmerresistance (<https://bitbucket.org/genomicepidemiology/kmerresistance>). Phenotypic AMR testing was performed using broth dilution according to EUCAST.

**Results:** A set of 96 German clinical *Campylobacter jejuni* isolates were analyzed with regard to their AMR profile. 85% of the isolates displayed reduced susceptibility to ampicillin with MHK<sub>24</sub> and all were predicted to harbor *oxa-61* or *oxa-184*. Susceptibility to ampicillin correlated with the absence of predicted  $\beta$ -lactamase genes. 53 out of 59 (90%) isolates with reduced susceptibility to ciprofloxacin showed a T86I mutation in *gyrA*. All susceptible isolates did not have predicted mutations conferring fluoroquinolone resistance. 87% of tetracycline resistant isolates harbored a putative *tetO* gene. 28% of the isolates were resistant to erythromycin with MIC<sub>2</sub> but only 14% showed a 23S rRNA mutation. In summary, concordance between genotype and phenotype of susceptibility to ampicillin, ciprofloxacin, tetracycline and erythromycin was 100%, 90%, 87% and 14%, respectively.

**Conclusion:** This study shows that phenotypic AMR testing correlates well with predicted resistance genes or mutations for  $\beta$ -lactams, fluoroquinolones and tetracyclines using standard prediction tools and databases. Prediction of tetracycline resistance does not correlate well with the AMR phenotype and therefore requires adjustments.

---

## 056-DKMP

### Detection and Quantification of a unique 16S ribosomal RNA allele variation in *B. anthracis* by combination of Fluorescence in situ hybridization, digital PCR and Nanopore Sequencing

\*F. Zimmermann<sup>1</sup>, P. Braun<sup>1</sup>, S. Mantel<sup>1</sup>, M. C. Walter<sup>1</sup>, K.

Aistleitner<sup>1</sup>, T. Sieper<sup>1</sup>, I. Stürz<sup>1</sup>, G. Grass<sup>1</sup>, K. Stoecker<sup>1</sup>

<sup>1</sup>Bundeswehr Institute of Microbiology, Medical Bio-Reconnaissance and Verification, Munich, Germany

*Bacillus anthracis* causes anthrax, a disease characterized by fatal septicemia in a broad range of mammals, including humans. It is part of the *B. cereus sensu lato* group of bacteria. While these species are closely related, they differ significantly concerning their phenotypes and most importantly their pathogenicity. For long, the 16S rRNA genes of *B. anthracis* were considered a poor target for

diagnosis of anthrax disease because of near identity to the other species of the *B. cereus sensu lato* group. Recently identified minor, yet specific differences (Hakovirta et al. 2016) make it now possible to harness the 16S rRNA genes to detect *B. anthracis* by molecular methods.

Based on a single nucleotide polymorphism (SNP) in a part of the *B. anthracis* 16S rRNA gene copies, we aimed at designing assays for the specific 16S rRNA based detection of *B. anthracis*. We further used our approach to quantify the *B. anthracis* 16S rRNA gene allele composition in 18 diverging strains of *B. anthracis* (A, B and C branch).

We developed a novel fluorescence in situ hybridization (FISH) assay for the specific detection of the *B. anthracis* 16S rRNA SNP using probes including locked nucleic acids (LNA). Microscopic results were further investigated with a new tetraplex droplet digital PCR approach. Overall allele distribution and validity of our assays were confirmed by nanopore long-read sequencing.

Our newly designed FISH and digital PCR assays can be applied for specific 16S rRNA based detection of *B. anthracis* versus its closest relatives. We found that FISH signal intensity with the *B. anthracis* specific probe varied widely between different *B. anthracis* strains. The digital PCR approach confirmed that 16S rRNA gene allele distribution is not consistent and that even total number of 16S rRNA genes differs. This result was verified using nanopore sequencing.

SNPs in multi copy genes like the 16S rRNA region can easily be overlooked by using conventional short-read whole genome sequencing and mapping approaches. If they are detected, i.e. by long-read sequencing, they can present an unexpected target for specific diagnostic approaches, as shown here for *B. anthracis*.

---

## 057-DKMP

### Detection and live/dead differentiation of *Legionella* species and *Pseudomonas aeruginosa* in water samples

N. Beaumart<sup>1</sup>, V. Herich<sup>1</sup>, B. Dobrindt<sup>1</sup>, \*T. Kuczius<sup>1</sup>

<sup>1</sup>Institute of Hygiene, University of Münster, University Hospital Münster, Münster, Germany

*Legionella* species and *Pseudomonas aeruginosa* have been frequently reported as pathogens contaminating water pipelines and dental chair units. Disinfections and decontamination procedures often lead to the desired result with a dramatic decrease of cultivable microorganisms. However, bacteria may change into a viable but not cultivable status (VBNC) showing no growth by culturing but being able to regain living conditions with re-growth and infectivity under specific conditions.

For identification and for clear live/dead differentiation we developed a real-time polymerase chain reaction (PCR) assay with the use of propidium monoazid (PMA). PMA permeates through damaged cell membranes of dead cells and binds covalently into DNA under blue light influence. A following PCR reaction is inhibited. In contrast, DNA of living cells will be amplified, because PMA is not able to penetrate intact bacterial membranes.

Specific primer pairs of the 16S rRNA gene and the elastase gene for *Legionella* species and *Pseudomonas aeruginosa* detection, respectively, were designed and PCR conditions were established for efficient and specific DNA amplification. No signals were identified with other accompanying bacterial

flora. Furthermore, the PMA concentration and the light impact were optimized using this technique. To each assay an internal amplification control was added comprising heat (99°C, 10 min) or isopropanol (70%) killed bacterial cells.

This technique can be used for rapid and sensitive differentiation of membrane damaged cells and cells with intact membrane structures and will be applied in success monitoring of disinfection procedures on basis of membrane damages such as quaternary ammonium disinfectants, chlorine and hydrogen peroxide.

---

#### 058-DKMP

Optimization of a Disc Diffusion Method for Antimicrobial Susceptibility Testing of *Clostridioides difficile*

\*S. Maurischat<sup>1</sup>, P. Witt<sup>1</sup>, C. Maneck<sup>1</sup>, M. Grobbel<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany

*Clostridioides (C.) difficile* is the major cause of antibiotic-associated diarrhoea in humans and animals. Broad spectrum antibiotics, such as cephalosporins and fluoroquinolones, are known to increase the risk for *C. difficile* infections (CDI) and have likely facilitated the epidemiological spread of highly virulent lineages (e.g. PCR-ribotype 027). The disc diffusion (DD) methodology proved to be a suitable and convenient alternative to the current gold standard agar dilution to test for antimicrobial susceptibility. Yet, DD has major drawbacks with regard to reproducibility and the unknown influence of anaerobic conditions on the test result. The aim of this study within the OHEJP IMPART (IMproving Phenotypic Antimicrobial Resistance testing) project is the establishment of a robust DD protocol for *C. difficile* that allows proposing cut-off values for resistance determination using DD.

Ten *C. difficile* isolates were chosen and tested for antimicrobial susceptibility against clarithromycin, metronidazole, moxifloxacin and tetracycline using an initial DD protocol based on prior publications and EUCAST recommendations. We investigated the effect of using different inoculum densities, media and anaerobic conditions during preparation to develop an optimized protocol. Using this protocol, we conducted intralaboratory repeatability and reproducibility tests and compared inhibition zone diameters (IZD) vs. minimum inhibitory concentrations (MICs) for eight antimicrobial classes and app. 500 isolates.

The different media had no significant effect on the IZD, but to reach confluent growth for most of the strains the turbidity level must be amended from EUCAST recommendations. The largest variance resulted from different anaerobic conditions. Applying the optimized protocol, the DD method proved to be highly reproducible. DD cut-off values will be proposed based on the evaluation of app. 500 isolates.

We found different anaerobic conditions was the most critical point for standardization of the DD testing of *C. difficile* and recommend an optimized protocol for a robust procedure. The correlation of IZD (based on the optimized protocol) and MICs will be presented.

---

#### 059-DKMP

Seeing is believing: Can molecular diagnosis guide individualized therapy in endocarditis?

J. Kikhney<sup>1</sup>, L. Kursawe<sup>1</sup>, S. Eichinger<sup>2</sup>, W. Eichinger<sup>2</sup>, S. Julia<sup>1</sup>, A. Wießner<sup>1</sup>, \*A. Moter<sup>1</sup>

<sup>1</sup>Charité - Universitätsmedizin Berlin, Biofilmzentrum, Institut für Mikrobiologie und Infektionsimmunologie, Berlin, Germany

<sup>2</sup>Hospital Bogenhausen, Department of Cardiac Surgery, München, Germany

#### Introduction

In Infective Endocarditis (IE), early diagnosis of the causative microorganism is crucial for correct antibiotic therapy and the patients' outcome.

#### Objectives

We studies the impact of biofilm formation in IE samples.

#### Materials & methods

We used Fluorescence *in situ* Hybridization (FISH) combined with 16S rRNA-gene PCR and sequencing to visualize and identify the infectious agents in native as well as prosthetic valves and to study their biofilm formation. The signal intensity of the fluorescence-labelled FISH probes correlates to a high ribosome content of the bacteria indicating metabolic activity at the time point of surgery. We developed a spacer FISH assay for the detection of the 16S-23S intergenic spacer region that is only present in actively transcribing cells to detect the activity of bacterial cells more precisely on a single cell level.

#### Results

FISH visualized bacteria in the heart valves ranging from single cells to highly organized biofilms. Interestingly, we found FISH positive bacteria in culture negative samples and samples from patients under antibiotic therapy. Using the spacer FISH we visualized positive microbial cells in heart valves of patients under adequate therapy. Preliminary data point to a correlation between the biofilm state and treatment inefficiency.

#### Conclusion

FISH/PCR not only allows timely identification of the pathogens in IE, but also biofilm-staging and visualization of the effect of antimicrobial therapy at time of surgery. The technique provides crucial information for successful targeted antibiotic therapy, and it might guide therapeutical decisions in relation to biofilm state in the future.

---

#### 060-DKMP

Multimodal analysis of *Escherichia coli* isolates from patients and carriers with EPISEQ® CS, a next-generation sequencing service for epidemiological surveillance

K. Vranckx<sup>1</sup>, \*L. Hauben<sup>1</sup>, S. Guedes<sup>2</sup>, K. De Raew<sup>1</sup>, K. De Bruyne<sup>1</sup>

<sup>1</sup>bioMérieux, Clinical Unit, Sint-Martens-Latem, Belgium

<sup>2</sup>bioMérieux, Marcy, France

Background:

In hospital environments, the presence of antibiotic resistant and/or virulent strains are of particular concern. In this work, we present a new, fast and high-resolution multimodal epidemiological analysis of WGS data with bioMérieux EPISEQ® CS that easily provides information on the relatedness, resistance and virulence of strains.

Materials/methods:

We analyzed WGS data of 22 *E. coli* publically available isolates from either patients with symptoms typical of an *E. coli* infection and asymptomatic carriers. This online application starts from raw reads with de novo assembly, and allows a multimodal approach for epidemiological surveillance including phylogenetic analysis based on whole genome multi-locus sequence typing (MLST), 7-gene MLST, resistome and virulome analysis.

#### Results:

After upload of the metadata and fastq.gz files, EPISEQ® CS allowed the analysis of the 22 *K. pneumoniae* isolates with a turnaround time of just over one hour. The analysis outcome provides insight in the quality of the different steps in the pipeline, and clustered all isolates together in the final phylogenetic tree. Five different STs were detected in the set, and four clusters of related strains, two clusters from asymptomatic carriers, one cluster of symptomatic patients and one mixed cluster. The virulome shows the unique presence of stx2 in the cluster of patients, confirming current knowledge on this virulence factor. The strains in the mixed cluster have an identical resistome and virulome suggesting a recent transmission even between carrier and patient.

#### Conclusions:

As WGS is also becoming feasible to smaller clinical laboratories, there is a need for automated and standardized analysis of these data. EPISEQ® CS is a fast, high-resolution, easy-to-use and automated WGS application, providing phylogenetic analysis, typing and resistome analysis. The phylogenetic analysis next to the quality information and uploaded metadata provides a useful overview to detect and allow the analysis of possible transmission events and will enhance rapid and accurate epidemiological surveillance in clinical environments.

#### 061-DKMP

Analysis of the transition process from an organ system-based to an alternative case-based teaching concept for medical students in the course of infection control, microbiology and virology at the Goethe University, Frankfurt am Main, Germany

\*J. P. W. Himmels<sup>1</sup>, C. Brandt<sup>1</sup>, J. Sterz<sup>2</sup>, M. Ruesseler<sup>2</sup>, V. A. J. Kempf<sup>1</sup>

<sup>1</sup>Goethe University Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt am Main, Germany

<sup>2</sup>Goethe University Frankfurt, Frankfurt Interdisciplinary Simulation Training, Frankfurt am Main, Germany

**Introduction:** Increasing challenges in infection control, microbiology, and virology, such as multi- and panresistant "emerging pathogens", are facing medical educators with the question of alternative teaching methods. Evidence supports that problem orientated learning is a superior form of education. The former organ system-based course in "infection control, microbiology, and virology" taught at the Goethe University Frankfurt was restructured towards a case-based teaching concept in 2016/17.

**Objectives:** The goal of the present study was to qualitatively analyze the subjective learning progress among students during the course of infection control, microbiology, and virology dependent on the teaching method used, and the evaluation of the longitudinal knowledge retention in physicians. Furthermore, claims of medical students or physicians and confounding factors were identified.

**Materials and Methods:** Forty-five medical students, of which 23 attended the former teaching concept and 22 the restructured format, were interviewed via focus groups. Nine physicians were interviewed individually. Guided interviews were performed to explore learning attitudes, perceptions and options to improve the learning environment. The interviews were anonymously transcribed and analyzed with the qualitative text analysis software "MAXQDA".

**Results:** Medical students and physicians preferred a teaching concept focussing on aspects relevant for clinicians irrespective of the study arm. The newly implemented case-based teaching method improved students' motivation and understanding of the subject. The predominance of the medical lecturer in the learning environment remained unaltered. Moreover, previously identified independent confounding factors (e.g., noise level, group size) were confirmed. Students of the organ system-based teaching method and physicians complained of progressive loss of specific knowledge over time.

**Conclusions:** The new a case-based teaching concept may improve the learning environment with the potential for an increased learning effectiveness. The long term loss of specific knowledge in our subject remains a matter of concern.

---

#### 062-DKMP

##### Synergy testing of antibiotic combinations to treat chronic *Pseudomonas aeruginosa* lung infections in cystic fibrosis patients

\*A. Schmidt<sup>1</sup>, T. Preis<sup>1</sup>, S. Schwarz<sup>1</sup>, A. Hector<sup>2,3</sup>

<sup>1</sup>Universitätsklinikum Tübingen, Medizinische Mikrobiologie und Hygiene, Tübingen, Germany

<sup>2</sup>Kinderspital Zürich, Pneumologie, Zürich, Switzerland

<sup>3</sup>Universitätsklinikum Tübingen, Kinderklinik, Tübingen, Germany

The dominant pathogen during chronic respiratory infections in cystic fibrosis (CF) is *Pseudomonas aeruginosa* (PSA). Although eradication of early-life PSA infections is successful in most cases, it remains the main pathogen in later life affecting 60-70% of adult patients with CF. One reason is the high intrinsic resistance of PSA to many antibiotic classes, but also its adaption to the environment and its growth in biofilms in the CF lung influence the antibiotic susceptibility. To reduce the amount of PSA during chronic infection, combinations of antibiotics from different classes were used. However until now there are not enough studies to prove that these combinations are more efficient. In our study we aimed to identify effective combinations of antibiotics under conditions that mimic the CF environment. We used different growth media in a broth microdilution method and in a biofilm model to test the susceptibility to antibiotics and antibiotic combinations most used in CF therapy and compared it to the routine measurement in our diagnostic department. In these experiments we used different laboratory PSA strains as well as an early and a chronic CF isolate and a multidrug resistant clinical isolate. We found that a PSA isolate from a chronic CF infection shows different MIC values in the standard measurement compared to the broth microdilution method. These differences were not detected for all other strains. Comparing minimal medium, mimicking the CF sputum environment, with standard medium the MIC for both CF isolates did not change. All other PSA isolates showed higher MICs. These MIC changes lead for tobramycin to MICs above the breakpoint for resistance. As expected when the bacteria were grown as biofilms even a twofold MIC was not able to eradicate the bacteria. Interestingly, colistin was not able to reduce the biofilm for all used strains, despite they all show very low colistin MICs in planktonic growth. The combination of tobramycin/ceftazidime showed a high

synergistic effect for all strains grown planktonically and as biofilms. In further experiments we want to use additional sequential isolates from CF patients to validate our findings.

## 063-DKMP

Colistin resistance in Gram-negative rods from urine specimens: prevalence and importance of *mcr* isoforms

\*A. Hoffmann<sup>1</sup>, M. Christner<sup>1</sup>, M. Lütgehetmann<sup>1</sup>, H. Rohde<sup>1</sup>

<sup>1</sup>Universitätsklinikum Hamburg-Eppendorf, Institut für medizinische Mikrobiologie, Virologie und Hygiene, Hamburg, Germany

**Background:** Colistin is an antibiotic of last resort to treat highly resistant Gram-negative organisms. In humans, colistin-resistance mainly develops by within host evolution during colistin treatment. Recently, however, a transmissible, plasmid-encoded colistin resistance gene (*mcr*) was described. Routine analysis of urine specimens using colistin-containing CNA-plates unexpectedly yielded growth of *Enterobacteriales* and *Pseudomonas aeruginosa*. **Objectives:** The main objective of this study to estimate the load and mechanism of colistin-resistant Gram-negative rods in a prospective collection of diagnostic urine specimens collected at the University Medical Center Hamburg-Eppendorf. **Methods:** From 06/2018 – 06/2019, roughly 30 000 urine specimens were subjected to microbiological analysis using Columbia blood -, UTI- and CNA-agar. Bacteria growing on CNA-plates were identified to the species level. Susceptibility was tested using a VITEK2 instrument and colistin gradient stripes. All isolates were tested for the presence of *mcr* using a newly designed qPCR specific for *mcr*-1 to *mcr*-6. **Results:** In total, 49 / 30.000 urine specimens grew Gram-negative bacteria on CNA-agar. Susceptibility testing identified colistin resistance (MIC > 2 mg/L; Median 4; SD 14 ) in 25 / 49 (*K. pneumoniae* (n=16); *E. coli* (n=4); *P. aeruginosa*, (n=4)). 25/25 were resistant to third generation Cephalosporins, 9/25 to Carbapenems and over 50% to fluoroquinolones. Intriguingly, qPCR detected *mcr* only in 3/25 isolates. 3/3 were positive for *mcr* 1 (3x *K. pneumoniae*), whereas *mcr*-2 to *mcr*-6 were not detected in any of this isolates. **Conclusion:** Primary colistin resistance in *Enterobacteriales* and *P. aeruginosa* is encountered in isolates from urine specimens at a low frequency. Resistance is predominantly independent from *mcr* 1-6. To the best of our knowledge, this is the first prospective collection identifying *mcr*-1 in human specimens in Germany and only the second detection of *mcr*-1 in Germany in human specimens so far. Ongoing work including whole genome sequencing is aiming on deciphering the molecular basis in *mcr* negative isolates.

## 064-DKMP

Performance and clinical impact of Rapid Antimicrobial Susceptibility Testing (RAST) on management of bloodstream infections in a tertiary care hospital

\*B. Berinson<sup>1</sup>, F. Olea<sup>1</sup>, M. Christner<sup>1</sup>, H. Rohde<sup>1</sup>

<sup>1</sup>Institute for medical Microbiology, Virology and Hygiene, Hamburg, Germany

**Introduction:** Fast and reliable antimicrobial susceptibility testing ensures early adequate therapy and may reduce the prolonged use of empiric broad-range antibiotics in gram-negative BSI. EUCAST has validated new breakpoints for short incubation disk diffusion testing directly from positive blood cultures, interpretable after 4, 6 and 8 hours (RAST).

**Objectives:** We aimed to evaluate the performance of RAST in comparison to the standard of care, VITEK2, and the

clinical effects of RAST on BSI management in our tertiary care center.

**Materials & methods:** RAST was implemented at our laboratory in addition to standard of care (SOC; Vitek2), i.e. availability of AST results a day after blood culture positivity. For all interpretable results categorical agreement (CA), concordant interpretation by SOC and RAST), very major error (VME, resistant by SOC, susceptible by RAST), major error (ME, susceptible by SOC, resistant by RAST) and minor error (mE, susceptible, increased exposure by SOC, resistant or susceptible by RAST) rates were calculated. To assess clinical impact of RAST, the proportion of patients receiving optimal antimicrobial therapy before and after communication of RAST was determined.

**Results:** For 97 positive blood cultures overall CA between RAST and SOC was 97.1 %; mE rate was 1.5 %, ME rate was 1.4 % and no VME was observed. A significant number of results (158/879) within the area of technical uncertainty were seen. Clinical impact of RAST was evaluated for 51 patients. In 90.2 % of these cases RAST was available after 4h, in 7.8 % after 6h and in 2 % after 8h. Optimal treatment was achieved in 41.2 % of the patients according to RAST and among these, RAST allowed an early escalation to a broad-spectrum antibiotic in 6 cases due to multi-resistant isolates. An unnecessary escalation based on RAST occurred in 17.6 % of the patients.

**Conclusion:** Our findings suggest that the implementation of RAST may be especially helpful for early treatment escalation in BSI caused by multi-resistant bacteria. RAST result communication should be integrated into structured antimicrobial stewardship programs to prevent inadequate or premature treatment adjustments.

## 065-DKMP

Comparison of three different methods for bacterial identification used in routine diagnostics with whole-genome based average nucleotide identity for *Roseomonas mucosa* identification

M. Trauth<sup>1,2</sup>, R. Bigge<sup>1</sup>, F. Gunzer<sup>3</sup>, B. Bunk<sup>4</sup>, W. W. Rudolph<sup>5</sup>, \*P. Schröttner<sup>1</sup>

<sup>1</sup>Technische Universität Dresden, Institut für Medizinische Mikrobiologie und Hygiene, Dresden, Germany

<sup>2</sup>Technische Universität Dresden, Klinik und Poliklinik für Anästhesiologie und Intensivmedizin, Dresden, Germany

<sup>3</sup>Technische Universität Dresden, Zentralbereich Krankenhaushygiene, Dresden, Germany

<sup>4</sup>Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany

<sup>5</sup>Technische Universität Dresden, Institut für Virologie, Dresden, Germany

The bacterial species *Roseomonas mucosa* is capable to cause infections in humans but is only rarely found during routine diagnostics. A main characteristic is the slow growth and the pink color of bacterial colonies. In most cases severe diseases such as bacteraemia, osteomyelitis and cellulitis are associated with *R. mucosa* (1). Immunosuppression and the use of catheter systems seem to be risk factors (1). To clarify the clinical significance of rare human pathogenic bacteria (such as *R. mucosa*), it is advisable to choose a prospective study approach. However, prior to study planning, it is necessary to know which is the best method for identification. For this reason, different routine methods (VITEK 2, MALDI-TOF MS and sequencing of 16S rRNA gene) were compared (2). Additionally, all genomes of the strains were sequenced on a HiSeq system and used to determine the Average Nucleotide Identity (ANI) (2). ANI is regarded as gold-standard of the molecular-based bacterial

species identification and was therefore used as reference method. A total of 9 isolates primarily identified as *R. mucosa* were included. Analyses using VITEK 2 revealed misidentifications (e.g. *Roseomonas gilardii*, *Rhizobium radiobacter* or *Sphingomonas paucimobilis*) (2). Eight of nine isolates were correctly identified as *R. mucosa* by sequencing the 16S rRNA gene. MALDI-TOF MS analyzes also showed reliable identification in eight out of nine isolates. However, one isolate repeatedly revealed score values below 2.0, indicating secure identification at the genus but not at the species level. With the help of ANI, however, it could be demonstrated that this strain actually belongs to the species *R. gilardii* and was misidentified as *R. mucosa* by sequencing the 16S rRNA gene (2).

#### References:

1. De I, Rolston KVI, Han XY. Clinical Significance of *Roseomonas* Species Isolated from Catheter and Blood Samples: Analysis of 36 Cases in Patients with Cancer. Clin Infect Dis. 2004;
2. Rudolph WW, Gunzer F, Trauth M, Bunk B, Bigge R, Schröttner P. Comparison of VITEK 2, MALDI-TOF MS, 16S rRNA gene sequencing, and whole-genome sequencing for identification of *Roseomonas mucosa*. Microb Pathog. 2019;

#### 066-DKMP

##### The Pan-Surfome of *Aspergillus fumigatus*

\*L. J. Jia<sup>1,2</sup>, T. Krüger<sup>1,2</sup>, O. Kniemeyer<sup>1,2</sup>, A. Brakhage<sup>1,2</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology, Department of Molecular and Applied Microbiology, Jena, Germany

<sup>2</sup>Institute of Microbiology, Friedrich Schiller University, Department of Microbiology and Molecular Biology, Jena, Germany

*Aspergillus fumigatus* is one of the most common airborne fungus that causes invasive mycoses in immunocompromised patients, and also allergic diseases in immunocompetent individuals. In both cases, the surface proteins mediate the first contact with the human immune system to evade immune responses or to induce hypersensitivity. The peptides exposed to the surface may be reasonable vaccine candidates or may be used for diagnostic purposes. Several methods have been established to study the surface proteome (surfome) of *A. fumigatus*, like trypsin shaving, glucanase treatment or formic acid extraction. Biotinylation coupled with LC-MS/MS identification is a particular efficient method to identify the surface exposed regions of proteins that could mediate the interaction with host. By biotinylation modification of proteins during the germination process we detected 314 surface proteins. In combination with other surfome data, about 500 surface proteins were identified. 150 proteins, including CcpA, Alp2, AspF1, DppV, were detected by at least two different methods. Using immunofluorescence microscopy, we confirmed the surface localization of several candidate proteins, including those without signal peptides. Collectively, our study generated a comprehensive data set of the *A. fumigatus* surfome. In addition, for many proteins it uncovers the regions, which are exposed to the surface of spores or hyphae. These surface exposed regions may represent antigenic epitopes that induce protective immune responses.

#### 067-DKMP

Effect of antibiotic exposure on the conjugative transfer of IncN *bla*VIM-1 carbapenemase plasmid in *Enterobacteriaceae*.

X. Li<sup>1</sup>, K. Kocer<sup>1</sup>, K. Heeg<sup>1</sup>, S. Boutin<sup>1</sup>, \*D. Nurjadi<sup>1</sup>

<sup>1</sup>Heidelberg University Hospital, Department of Infectious Diseases (Microbiology and Hygiene), Heidelberg, Germany

#### Introduction

Antibiotics provide a critical selective pressure for the horizontal transfer of antibiotic resistance and is a major facilitator of the emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE). In this study we investigated the effect of various antibiotics on the frequency of conjugative transfer of *bla*VIM-1 plasmids.

#### Method

We investigated the effect of various antibiotics on the conjugative efficiency of five isolates with *bla*VIM-1 carrying plasmids from patients colonized/infected with multiple CRE species by liquid mating using *E. coli* strain J53 as recipient. Success of conjugation was confirmed by WGS. Conjugation frequency was determined by quantification of transconjugants growing on a selective medium.

#### Results

WGS confirmed conjugative transfer of the full IncN2 pVIM-1\_KE3510 plasmid to the recipient strain. Beta-lactam antibiotics, aminoglycosides and trimethoprim-sulfamethoxazole did not increase conjugation frequency. Only sub-inhibitory concentrations of ciprofloxacin were able to increase conjugative transfer by 10-fold. Similar effects were observable with levofloxacin and moxifloxacin as well as using other donors with identical *bla*VIM-1 plasmids. Our observations suggested a species-independent and plasmid specific effect as no such effect could be observed when using different *bla*VIM-1 carrying donor strains. This effect may be mediated by co-resistance genes present on the same plasmid and warrant further investigation.

#### Conclusion

Quinolone exposure can influence conjugation frequency of certain *bla*VIM-1 plasmids and our findings suggest that this effect may be attributed to the presence of co-resistance genes in the plasmids. Identification of "high-risk" genes for plasmid transfer may help to evaluate and optimize preventive measures in the clinical setting.

#### 068-DKMP

##### Comparison of different methods used in routine diagnostics with Average Nucleotide Identity and Digital DNA-DNA hybridization for identification of *Acinetobacter radioresistens*

\*R. Bigge<sup>1</sup>, F. Gunzer<sup>2</sup>, B. Bunk<sup>3</sup>, W. W. Rudolph<sup>4</sup>, P. Schröttner<sup>1</sup>

<sup>1</sup>TU Dresden, Institut für Medizinische Mikrobiologie und Hygiene, Dresden, Germany

<sup>2</sup>Universitätsklinikum Carl Gustav Carus, Zentralbereich Krankenhaushygiene/Umweltschutz, Dresden, Germany

<sup>3</sup>Leibniz-Institut DSMZ-Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany

<sup>4</sup>TU Dresden, Institut für Virologie, Dresden, Germany

**Introduction:** The bacterial species *Acinetobacter radioresistens* was first described in the 1980s. In one study, *A. radioresistens* could be detected within up to 12% of the population proving that these bacteria are part of the natural skin flora of humans. However, detection of *A. radioresistens* from clinical specimens has been rarely reported. This may be due to the fact that *A. radioresistens* was considered non-pathogenic. Recent improvements in routine diagnostics



have led to an increase in detection of isolates. The first clinical case detecting a pathogenic potential for *A. radioresistens* dates back to the early 2000s. Since then a number of case reports has been published.

**Objectives:** Isolates have to be collected in a prospective study in order to further elucidate which methods provide a secure species identification. Different routine methods of bacterial diagnostics have to be compared.

**Materials and methods:** From 2013 to 2019, a total of 13 isolates of *A. radioresistens* were collected from the clinical routine diagnostics at the Institute for Medical Microbiology and Hygiene of the TU Dresden, Germany. Investigations were performed with VITEK 2, MALDI-TOF MS and sequencing of 16S rRNA and *rpoB* gene. In addition, whole genomes sequences of all strains were obtained using Illumina NextSeq. Based upon genome sequencing results Average Nucleotide Identity and Digital DNA DNA Hybridization are currently considered as molecular gold standard for bacterial species identification. For this reason, both were used as reference methods in this study but also compared to each other.

**Results:** The species *A. radioresistens* was securely identified in all isolates with ANI, dDDH as well as MALDI-TOF MS and 16S rDNA/*rpoB* gene sequencing. The identification using VITEK 2, however led to ambiguous results, affecting both species and genus.

**Conclusion:** MALDI-TOF MS may be considered the most suitable method for screening of *A. radioresistens*. Results are generated quickly and the species is securely identified. In case of questionable results, sequencing of the 16S/*rpoB* gene or a determination by ANI values or dDDH should be performed.

---

## 069-DKMP

### Comparison of two PCR tests for the detection of *Aspergillus* spp. in bronchoalveolar lavage fluid

\*L. D. Frielinghaus<sup>1</sup>, T. Kautz<sup>1</sup>, M. Luetgehetmann<sup>1</sup>, H. Rohde<sup>1</sup>, M. Christner<sup>1</sup>

<sup>1</sup>University Medical Center Hamburg Eppendorf, Medical Microbiology, Virology and Hygiene, Hamburg, Germany

Invasive aspergillosis (IA) is the most common mold infection in immunocompromised patients and associated with significant mortality. Current guidelines encourage the use of *Aspergillus* PCR in bronchoalveolar lavage fluid (BAL) to improve the diagnostic yield. We have compared two *Aspergillus* PCRs, the commercially available Bruker Fungiplex *Aspergillus* IVD PCR (CA-PCR) and a laboratory developed qPCR published by Walsh et al. 2011 (LD-PCR), in BAL samples sent to our laboratory for galactomannan testing (GM) and fungal culture.

Surplus material from clinical samples was prospectively stored at -80 °C. All 218 available GM positive and 70 arbitrarily selected GM negative samples were subjected to bead beating and automated nucleic acid extraction. DNA extracts were subjected to single CA-PCR testing according to manufacturer's instructions or to duplicate LD-PCR testing following an in-house protocol previously validated with spiked samples.

Overall agreement between CA- and LD-PCR was 87.5 % with similar positivity rates in culture positive (80.0 % and 75.6 %) and culture negative (22.2 % and 22.2 %) as well as in GM positive (39.0 % and 37.6 %) and GM negative

samples (7.1 % and 8.6 %). Cq-values from 71 samples which tested positive by both PCRs were found to be strongly correlated ( $r(69) = 0.92$ ,  $p < 0.001$ ) with slightly lower Cq values obtained with LD-PCR. Cq-values from both PCRs moderately correlated with GM levels ( $r(83) = -0.45$ ,  $p < 0.001$  for CA-PCR and  $r(80) = -0.51$ ,  $p < 0.001$  for LD-PCR).

Satisfactory agreement was found between two PCR tests when applied to BAL samples. In concordance with previous studies, PCR showed higher positivity rates than fungal cultures. The staggering rate of 51.8 % of GM positive samples which could neither be confirmed by culture nor PCR-testing fuels recent concerns about the specificity of GM in lower respiratory tract secretions and mandates judicious assessment of test utilization, especially in populations at lower risk for IA.

---

## 070-DKMP

Care duties, support systems and career challenges among medical microbiologists in Germany

### Results of the "Family and career within microbiology" survey conducted by the Young German Society for Hygiene and Microbiology (yDGHM)

\*K. Last<sup>1</sup>, C. M. Koch<sup>2</sup>, V. Schwierzeck<sup>3</sup>, S. L. Becker<sup>1</sup>, J. Forster<sup>4</sup>, C. Papan<sup>1</sup>, N. Jazmati<sup>5</sup>

<sup>1</sup>Institute of Medical Microbiology and Hygiene, Saarland University Medical Center, Homburg, Germany

<sup>2</sup>Institute for English and American Studies, Philipps University Marburg, Marburg, Germany

<sup>3</sup>Institute of Hygiene, University Hospital Münster, Münster, Germany

<sup>4</sup>Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Germany

<sup>5</sup>Labor Dr. Wisplinghoff, Cologne, Germany

**Introduction:** Approximately 65% of all medical graduates in Germany are female, yet, leading positions within the hospital setting are disproportionately more often held by men. One reason for this representation gap in high rank positions are family and care duties which are most prevalent for women at early career stages. Nevertheless, care duties are not only restricted to child care duties. Other periods in life may also be filled with care duties regarding parents, siblings or other family members.

**Objectives:** To estimate the overall burden of care duties among medical microbiologists in Germany, to picture the current infrastructure of work-place related support systems and to localize specific hurdles for parents and care-givers to engage in career-relevant activities such as research and scientific conferences.

**Materials/methods:** A cross-sectional web-based survey was conducted among members of the DGHM by using the online platform LimeSurvey (LimeSurvey GmbH, Hamburg, Germany). Eligibility was irrespective of specialist training status, hierarchic position and training background (medical or biological). Participants were asked to answer 37 questions. Invitation to the survey was disseminated via the official newsletters of yDGHM and DGHM.

**Preliminary Results:** The survey is currently online (<http://survey-wbs.imib.rwth-aachen.de/survey/index.php/331532?lang=de>) and is scheduled to close on November 17th 2019. A full analysis of the survey will be conducted subsequently and results will be presented during the conference.

---

## 071-DKMP

Investigation of the Bovine mastitis causing bacterial strains and Confirmation of its Virulence and Zoonotic Potential

\*F. Adnan<sup>1</sup>, A. Jalil<sup>1,2</sup>

<sup>1</sup>National University of Sciences and Technology (NUST), Industrial Biotechnology, ASAB, Islamabad, Pakistan

<sup>2</sup>National University of Sciences and Technology (NUST), Islamabad, Pakistan

Bovine mastitis is the inflammation of the mammary glands due to physical injury or microbial infection. It is one of the most common and expensive disease of dairy industry resulting huge economic loss due to reduced milk production. Mastitis can be further classified into clinical and sub-clinical types based on the degree of inflammation. In this study, prevalence of mastitis among cows and buffalos was investigated in the Rawalpindi district of Pakistan. The investigative parameters included in this study were herd size, sanitary conditions, management system, and hygiene of cows and buffalos. After confirmation of Mastitis with surf field mastitis test (SFMT), prevalence of mastitis causing bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Klalsiala* etc.) was analysed. *E. coli* isolates from bovine mastitis were classified into main phylogenetic groups by triplex-PCR based on the presence of the *chuA* and *yjaA* genes and the DNA fragment TSPE4.C2. *E. coli* strains were further screened for their antibiotic resistance and extended spectrum beta lactamases (ESBLs) production. Furthermore, virulence associated genes and toxin genes were also investigated by multiplex PCR. *In vitro* pathogenicity assays were performed for the estimation of disease causing potential of selected isolates. *In vitro* pathogenicity assays including biofilm formation, Congo red binding assay, growth in human urine, swimming and swarming motility, and hemolytic assay were performed for the estimation of the virulence and zoonotic potential of the selected isolates.

## 072-DKMP

**Isothermal nucleic acid amplification as a versatile tool for point-of-care hygiene monitoring**

\*S. Kersting<sup>1</sup>, E. Ehrentreich-Förster<sup>1</sup>, M. von Nickisch-Roseneck<sup>1</sup>

<sup>1</sup>Fraunhofer IZI-BB, Potsdam, Germany

Introduction:

The use of nucleic acid amplification techniques has significantly improved the specificity and sensitivity of diagnostic tests for the detection of pathogenic organisms. However, the PCR usually remains laboratory-bound and is not available for on-site tests. Alternatively, isothermal nucleic acid amplification can be utilized.

Objectives:

In the past, several assays and systems for the detection and characterization of numerous pathogenic organisms based on isothermal nucleic acid amplification have been established in our group. Alternative read-out options included naked-eye (turbidity and colorimetry), spectrometric, fluorometric and lateral-flow dipstick formats were tested and optimized to offer maximum flexibility for system integration.

Results:

We were able to demonstrate the specific and highly sensitive detection of pathogens in less than 20 minutes. A special primer/probe – system allows the direct detection of the amplification product on lateral-flow-strips. The reaction can work instrument-free and the result is given in a format which can be read out by the untrained eye. Further

application-oriented systems include assays for microfluidic lab-on-chip devices and the usage in rudimentary diagnostic equipment.

Conclusion:

The flexibility of isothermal nucleic amplification offers new possibilities for point-of-care tests and may complement existing methods and technologies as PCR and immunochromatographic on-site tests.

## 073-DKMP

Evaluation of the new Bruker MALDI Biotyper sirius MALDI-TOF MS system in comparison to microflex LT

\*A. Pranada<sup>1</sup>, T. Wenzel<sup>2</sup>, A. Sägers<sup>1</sup>, C. Rompf<sup>1</sup>, M. Kostrzewa<sup>2</sup>

<sup>1</sup>MVZ Dr. Eberhard & Partner Dortmund (ÜBAG), Mikrobiologie, Dortmund, Germany

<sup>2</sup>Bruker Daltonik GmbH, Bremen, Germany

**Introduction:** All currently available commercial systems for MALDI-TOF MS based microorganism identification work in positive ion mode. Recently applications for negative ion mode – like detection of colistin resistance in bacteria – have been published. The MALDI Biotyper sirius system comprises a mass spectrometer capable of working in positive as well as negative ion mode.

**Objectives:** We have evaluated the performance of the new MALDI Biotyper sirius system for standard identification in positive ion mode in comparison to a microflex LT/SH system.

**Materials and Methods:** N=520 bacterial isolates and yeasts of 87 different species from clinical routine were identified by the MALDI Biotyper sirius system as well as the microflex LT/SH system in parallel. Identification was performed using the Direct Transfer procedure (DT) and subsequently extended Direct Transfer (eDT) and extraction (Ext) procedure if the log(score) values were below 2.0. On both systems the latest version of the MBT Compass IVD software was used.

**Results:** Of the N=520 isolates the MBT sirius system identified 509 (97.9%) at high confidence level with log(score) values  $\geq 2.0$  in the combined workflow (DT+eDT+Ext) and 520 (100%) at low confidence level (log(score) $\geq 1.7$ ). For the microflex LT/SH system the results were 516/520 (99.2%) and 519/520 (99.8%), respectively. With Direct Transfer only, the MBT sirius system identified 484/520 (93.1%) at high confidence level and the microflex LT/SH system 498/520 (95.8%). None (0%) of the isolates were misidentified.

**Conclusion:** Both, the new MBT sirius system as well as the MBT standard system, reliably identified the set of 520 clinical strains. In the combined workflow of DT+eDT+Ext, both systems achieved >97% identifications to species level at high confidence level without any misidentifications. Even with only DT procedure more than 93% of the isolates yielded log(score) values at high confidence level. The MBT sirius is a reliable system for microorganism identification in routine laboratories. In combination with the possible applications of the integrated negative ion mode it could further improve the workflow in microbiology in the future.

## 074-DKMP

## German physicians are largely unaware of the new EUCAST definitions of S, I and R and the consequences thereof for antibiotic prescription

\*N. Wantia<sup>1</sup>, S. G. Gatermann<sup>2</sup>, K. Rothe<sup>1</sup>, R. Laufenberg<sup>3</sup>

<sup>1</sup>Technische Universität München, Institut für Medizinische Mikrobiologie, Immunologie und Hygiene, München, Germany

<sup>2</sup>Ruhr-Universität Bochum, Institut für Hygiene und Mikrobiologie, Bochum, Germany

<sup>3</sup>MVZ SYNLAB Leverkusen GmbH, Mikrobiologie, Leverkusen, Germany

**Objective:** Since January 1st 2019, the new EUCAST definitions of susceptibility testing categories S, I and R are valid. The new definitions and breakpoints reflect the need for correct exposure of the microorganism to the antimicrobial agent at the site of infection. Microbiological departments working with EUCAST rules are obliged to implement the new definitions and teach physicians how to interpret the modified antibiograms. We conducted a nationwide web-based survey reaching across disciplines. We questioned in our survey, if German physicians know the new definitions of S, I and R, if they feel well-informed about the changes, and if they are aware of the consequences for clinical practice. **Method:** We performed a web-based survey using LamaPoll. The survey targeted clinicians across all disciplines and was active from May 9th to July 30th 2019. 902 questionnaires were included in the analysis, and 852 questionnaires were completely answered. Participating physicians were mainly employed at hospitals (79.3%) and had completed specialist training (86.1%). 59.8% were male, and the predominant specialty was internal medicine (50.6%). **Results:** Of all participants, 45.7% did not know that there was a change in the definitions and 65.4% did not feel well-informed about the changes. In a knowledge quiz, substantial knowledge gaps were apparent, e.g., only 70.7% correctly named the new definition of I (i.e., "susceptible, increased exposure"), and merely 41.8% knew that some pathogen/antibiotic-combinations are no longer reported S but I to emphasize the need for a higher treatment dose. The participants reached an average of 5.5 points of 11 possible. Test performance mainly depended on whether participants were aware of the changes in EUCAST 2019 (in average, 7.0 compared to 3.8 points), whereas geographic region, age or sex had no clear impact. Worst results were achieved by those physicians who work in a practice. **Conclusion:** Though our survey might not be representative, it clearly shows that there are considerable knowledge gaps in Germany. This might severely hamper optimal treatment of infectious diseases, thus further education is strongly needed.

## 075-DKMP

Population-wide antibiotic profiling of human *L. monocytogenes* isolates from Germany identifies phylogenetic clades with reduced cephalosporine resistance

\*M. Fischer<sup>1</sup>, A. Flieger<sup>1</sup>, S. Halbedel<sup>1</sup>

<sup>1</sup>Robert Koch-Institute, German-Austrian Binational Consiliary Laboratory for *Listeria*, FG11 - Enteropathogenic Bacteria and *Legionella*, Wernigerode, Germany

*Listeria monocytogenes* is an important food-borne pathogen mostly associated with milk products, but also occurring on meat and vegetables and is the causative agent for listeriosis, an illness ranging in its symptoms from gastroenteritis to septicemia, meningoencephalitis and abortion in pregnant females. Case numbers are increasing during the last decade with almost 700 notified cases in 2018 in Germany. Fatality rates range between 7 to 30 %, making listeriosis one of the most fatal gastrointestinal infections. *L. monocytogenes* is intrinsically susceptible to a variety of

antibiotics, but the number of reports on antibiotic-resistant *L. monocytogenes* isolates is steadily increasing.

Monitoring the development of antibiotic resistance and identifying genetic determinants involved in resistance formation is of utmost importance. The German consiliary laboratory for *Listeria* collects clinical isolates from all over the country and sequences their genomes for phylogenetic subtyping (1). Guided by a genome sequence based analysis of the *L. monocytogenes* population structure, we created a collection of ~500 clinical *L. monocytogenes* isolates constituting a representative subset of the entire *L. monocytogenes* population. This collection was screened for susceptibility towards clinically relevant antibiotics. All isolates were sensitive against penicillin and gentamycin and were resistant towards ceftriaxone. Intrinsic resistance towards cephalosporins is a known feature of *L. monocytogenes*, however, reduction of cephalosporine resistance level was observed in certain phylogenetic clades. We employed different comparative genomic approaches to identify alleles or mutations explaining this reduced cephalosporine resistance observed in these clades. Among the observations made was a variation of the *Imo0441* allele, encoding penicillin binding protein PBP B3, which was exclusively associated with cephalosporine sensitivity. This is in good agreement with previous results demonstrating a role for PBP B3 in cephalosporine resistance of *L. monocytogenes* (2) and *Bacillus subtilis* (3). Current results of this ongoing research will be presented.

---

(1) Halbedel, S., Prager, R., Fuchs, S., Trost, E., Werner, G., & Flieger, A. (2018). Whole-genome sequencing of recent *Listeria monocytogenes* isolates from Germany reveals population structure and disease clusters. *Journal of clinical microbiology*, 56(6), e00119-18.

(2) Krawczyk-Balska, A., & Markiewicz, Z. (2016). The intrinsic cephalosporin resistome of *Listeria monocytogenes* in the context of stress response, gene regulation, pathogenesis and therapeutics. *Journal of applied microbiology*, 120(2), 251-265.

(2) Wei, Y., Havasy, T., McPherson, D. C., & Popham, D. L. (2003). Rod shape determination by the *Bacillus subtilis* class B penicillin-binding proteins encoded by *pbpA* and *pbpH*. *Journal of bacteriology*, 185(16), 4717-4726.

## 076-DKMP

An automated system for colony counting and classifying bacteria

\*J. Beck-Nafz<sup>1,2</sup>, A. Klimovich<sup>2</sup>, R. Preyer<sup>2</sup>, D. Drissner<sup>1</sup>

<sup>1</sup>Albstadt-Sigmaringen University, Microbiology, Food Hygiene and Consumer Health Protection, Department of Life Sciences, Sigmaringen, Germany

<sup>2</sup>AID/GenID Diagnostika, Strassberg, Germany

Colony counters can be used in the food and pharmaceutical industries to replace manual counting, i.e. the counting process of agar plates, through automation. Here, we present the procedure of validation of an automated, image processing based system, the Colony Counter BAC051806013 (AID/GenID Diagnostika, Straßberg, Germany). The objective of our study was to give recommendations regarding the light and count settings of the counter, under which the best counting results can be achieved. We performed several test series using the counter with different bacterial species and agar plates and established specific adjustment recommendations regarding

count settings like intensity, size, roundness, object dimension and object intensity for optimal detection of various *Legionella* species and the *Bacillus cereus* group. The Colony Counter presented in this case study can make a significant contribution towards optimizing the process of colony counting on agar plates in microbiological testing in various industries including medical diagnostics, pharmaceutical microbiology as well as food quality and safety control.

## 077-DKMP

Survey on antimicrobial resistance patterns in *Aeromonas* spp. from German patients

\*K. Schwartz<sup>1</sup>, J. A. Hammerl<sup>1</sup>, E. Strauch<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany

**Keywords:** *Aeromonas* spp., clinical isolates, antimicrobial resistance pattern, broth microdilution, disk diffusion

*Aeromonas* spp. bacteria are present in aquatic environments and can be isolated from insects, domestic animals, and foods of animal or plant origin. As opportunistic pathogens, they are associated with human infections most often manifested by gastroenteritis, wound infection or septicemia. In spite of sometimes severe to life-threatening infections in immunocompromised hosts, no data on antimicrobial susceptibility of German clinical *Aeromonas* isolates exist. Given the increasing superannuation of the German population, the study aimed to investigate antimicrobial resistance patterns in *Aeromonas* isolates from German patients.

We selected 61 well documented clinical strains associated with intestinal and extraintestinal infections between 2016 and 2019. Antimicrobial susceptibility was determined by broth microdilution and disk diffusion according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

The majority of German clinical *Aeromonas* isolates were susceptible to most of the 28 antimicrobial agents tested. Non-susceptibility was found towards aminopenicillins. In addition, a noticeable proportion of strains showed intermediate to full resistance to carbapenems, quinolones, polymyxins and/or cephamycins. Non-susceptibility towards third- or fourth-generation cephalosporins, fluoroquinolones, folate pathway inhibitors, tetracyclines, phenicols and macrolides, respectively, was sporadically observed.

Antimicrobial agents recommended for treatment of *Aeromonas* infections were found to be effective *in vitro*. However, the occurrence of sporadic resistances towards third- or fourth-generation cephalosporins as well as fluoroquinolones highlights the need for systematic monitoring of antimicrobial susceptibility in *Aeromonas* spp. in Germany.

## 078-MCBP

Novel C-methyltransferase activities: Methylation and simultaneously five-ring cyclisation is a prerequisite for the biosynthesis of the sesquiterpene sodorifen of *Serratia plymuthica*

N. Magnus<sup>1</sup>, M. C. Lemfack<sup>1</sup>, S. von Reuß<sup>2</sup>, D. Domik<sup>1</sup>, M. Kai<sup>1</sup>, T. Weise<sup>1</sup>, \*P. Ruciaka<sup>1</sup>, B. Piechulla<sup>1</sup>

<sup>1</sup>University of Rostock, Biochemistry, Rostock, Germany

<sup>2</sup>Université de Neuchâtel, Institut de chimie, Neuchâtel, Switzerland

Microorganisms are able to produce a plethora of secondary metabolites for their defense and survival. Among them, terpenoids are a major class of biologically active substances. They are mostly synthesized by *Streptomyces* species, while other bacterial clades are less well known to produce these secondary metabolites. The rhizobacterium *Serratia plymuthica* (Gamma Proteobacteria, Enterobacteriaceae) is capable to synthesize a unique and unusual polymethylated, bicyclic sesquiterpene named sodorifen (C<sub>16</sub>H<sub>26</sub>) [1, 2]. This compound was found to possess toxicity against colorectal carcinoma cell lines and its production can be significantly up regulated during co-cultivation with other microorganisms [3, 4]. Transcriptome analysis and genome sequencing revealed a cluster of four genes involved in the biosynthesis of sodorifen (sodorifen cluster). These genes encode a terpene cyclase, C-methyltransferase, DXP synthase and IPP isomerase. The biosynthesis of sodorifen was elucidated recently [4]. Interestingly, unlike other terpene synthases, this *S. plymuthica* terpene cyclase (S.p. SODS) does not accept the canonical C<sub>15</sub> substrate farnesylpyrophosphate (FPP), but a C<sub>16</sub> pre-sodorifen pyrophosphate. The latter is formed by the C-methyltransferase of the sodorifen cluster (S.p. FPPMT) which not only methylates the FPP substrate but also induces a first cyclization reaction [5]. Subsequently, this methylated, cyclized FPP (pre-sodorifen-pyrophosphate) is the substrate of the terpene cyclase which performs additional cyclisation cascades to finally produce sodorifen. To our knowledge, this is the first methyltransferase described which catalyzes a methylation with simultaneous five-ring cyclisation of a prenyl pyrophosphate substrate during terpene biosynthesis.

[1] Kai et al., (2010) Appl Microbiol Biotechnol.

[2] von Reuß et al., (2010) Angew. Chem.

[3] Kai and Piechulla, (2018) FEMS Microbiol Lett.

[4] Schmidt, (2017) Sci. Rep

[5] von Reuß et al., (2018) JACS.

## 079-MCBP

Transcription factors and chromatin-modifiers involved in sexual development of *Sordaria macrospora*

\*R. Lütkenhaus<sup>1</sup>, J. Breuer<sup>1</sup>, M. Nowrousian<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Molecular and Cellular Botany, Bochum, Germany

**Introduction and Objectives:** The model fungus *Sordaria macrospora* belongs to the ascomycetes and is ideal to investigate multicellular development. In this study, we focused on transcription factors and chromatin-modifiers to investigate the regulatory networks of fruiting body development. Comparative transcriptomics of three different ascomycetes and RNA sequencing of developmental mutants from *S. macrospora* led to identification new putative developmental genes. Genes of interest were either upregulated in young fruiting bodies in all three ascomycetes or downregulated in protoperithecia of developmental mutants. The chromatin-modifiers encoded by *spt3* and *scm1* were chosen by comparative genomics. Two transcription factor genes, *SMAC\_03952* and *asm2* (*ascospore maturation factor 2*), were downregulated in the protoperithecia of two different sterile mutants.

**Methods:** To investigate the function of the genes we constructed knockout strains. For characterization studies, the deletion strains were complemented with GFP-tagged constructs, which enabled localization studies. For RNA-Seq, strains with a developmental phenotype were chosen.

**Results:** Three of four knockout strains showed a developmental phenotype as single knockout except for SMC1, which seemed not to be involved in fruiting body formation. However, its function is redundant with three other chromatin-modifiers, since multiple deletion strains showed reduced fertility up to sterility. Deletion of the other chromatin-modifier SPT3, subunit of the SAGA complex, resulted in the most conspicuous phenotype.  $\Delta$ spt3 forms only few unpigmented protoperithecia. Also sterile but forming deformed perithecia with ascus precursors is the  $\Delta$ 3952 mutant strain. The knockout strain of *asm2* showed defects in ascospore maturation hence is not essential but developmentally relevant. RNA from  $\Delta$ asm2,  $\Delta$ 3952 and  $\Delta$ spt3 as well as from wild type was isolated for RNA-seq. The RNA-Seq data revealed SPT3 as regulator of a vast number of genes, whereas SMAC\_03952 and ASM2 regulated more genes in perithecia than in sexual mycelium. Analysis of the data will help to close gaps in the regulatory network behind fruiting body formation.

#### 080-MCBP

##### The [N]-shuttle catalyzed by DcuA and AspA is regulated in *E. coli* by the nitrogen-regulator protein PII

\*C. Schubert<sup>1</sup>, A. Strecker<sup>1</sup>, G. Uden<sup>1</sup>

<sup>1</sup>Johannes Gutenberg-University, Microbiology and Wine Research, Mainz, Germany

The alternative C<sub>4</sub>-dicarboxylate transporter DcuA of *E. coli* is required together with aspartase AspA for aerobic growth with L-aspartate as a nitrogen source [1]. By the combined action of DcuA and AspA L-aspartate was taken up by the bacteria and fumarate was excreted in equimolar amounts. L-Aspartate was taken up in amounts required for nitrogen but not for carbon metabolism. Therefore, DcuA catalyzes an L-aspartate/C<sub>4</sub>-dicarboxylate antiport which serves as a nitrogen shuttle for nitrogen supply without net carbon supply [1]. This ammonium supplying [N]-shuttle was regulated by the nitrogen regulator protein PII. PII in presence of 2-oxoglutarate stimulated AspA (L-aspartate deaminating reaction) resulting in the acquisition of ammonium under nitrogen-limiting conditions. Ammonium is then assimilated by the main nitrogen assimilation GS-GOGAT pathway of *E. coli*. Growth experiments show that L-aspartate is a preferred nitrogen source of *E. coli* which agrees with the regulation by the central N-regulation pathway [2] and the physiological role of L-aspartate as a N-source for *E. coli* in the bovine intestine [3].

[1] Strecker *et al.*, 2018 Molecular Microbiology 00(0), 1–11

[2] Schubert *et al.*, submitted

[3] Bertin *et al.*, 2018 Environmental Microbiology Vol. 20 No. 18

#### 081-MCBP

##### Biogenesis of Fe-S cluster proteins of *Vibrio cholerae*

\*J. L. Hau<sup>1,2</sup>, \*K. Goebel<sup>1</sup>, C. Toulouse<sup>1,2</sup>, J. Pfannstiel<sup>2</sup>, G. Fritz<sup>1</sup>, J. Steuber<sup>1</sup>, \*S. Agarwal<sup>1</sup>

<sup>1</sup>Hohenheim, microbiology, Stuttgart, Germany

<sup>2</sup>University Hohenheim, Stuttgart, Germany

The Na<sup>+</sup> - ion translocating NADH:quinone oxidoreductase (NQR) from *Vibrio cholerae* is a membrane bound respiratory enzyme which harbors flavins and Fe-S clusters as redox centers. The NQR is the main producer of sodium motive force (SMF) and drives energy-dissipating processes such as flagellar rotation, substrate uptake, ATP synthesis and

cation-proton antiport. In our proteome study [1], we compared the abundance of proteins in the *V. cholerae* O395N1 (reference strain) with its mutant  $\Delta$ nqr derivative strain, where the six structural genes *nqrABCDEF* of the operon encoding for the membrane bound NQR respiratory complex were deleted. The proteomic study revealed that most of the iron homeostasis proteins were present in similar abundances in both strains. These included proteins predicted to participate in the assembly of Fe-S clusters, in the transport and storage of iron, and in the binding and transport of heme. However, interestingly, some of these proteins were differentially regulated. The genome predicts that *V. cholerae*, unlike *E. coli* which harbors the Isc, Suf and Csd-Suf hybrid systems [2] for Fe-S assembly biogenesis, possesses only a single Isc system for assembly of Fe-S clusters. The proteins predicted to participate in the biosynthesis of Fe-S clusters were present in similar abundance in the *V. cholerae* wt and  $\Delta$ nqr strains, indicating constitutive expression of these essential proteins in the two strains. Even though *V. cholerae* does not encode an additional Fe-S cluster assembly system, the presence of iron responsive regulators that are responsible for tight Fe-S homeostasis regulation point towards Fe-S assembly via different routes which may replace a part of the Isc machinery under stress conditions. We also show that the *nqr* operon comprises, besides the structural *nqrABCDEF* genes, the downstream *apbE* and *nqrM* genes on the same operon, which represent a flavin insertase (ApbE) and a putative assembly factor (NqrM) [3]. The possible pathway of FeS insertion in NQR is discussed.

#### References:

[1] C Toulouse *et al* J Bacteriology **200**; 761-17, **2018**

[2] B Roche *et al* Biochim Biophys Acta **1827**; 455–469, **2013**

[3] VA Kostyrko *et al* J Bacteriology **198**; 655–663, **2015**

#### 082-MCBP

##### Towards understanding the link between the ABC transporter EsIABC and lysozyme resistance in *L. monocytogenes*

\*J. Rismondo<sup>1,2</sup>, M. Yacoub<sup>2</sup>, A. Wadhawan<sup>2</sup>, M. S. Dionne<sup>2</sup>, A. Gründling<sup>2</sup>

<sup>1</sup>Georg-August-Universität Göttingen, Department of General Microbiology, Göttingen, Germany

<sup>2</sup>Imperial College London, Medicine, London, United Kingdom

The human pathogen *Listeria monocytogenes* is intrinsically lysozyme resistant. Lysozyme functions by hydrolysing the peptidoglycan (PG) layer of bacteria. The PG deacetylase PgdA and PG O-acetyltransferase OatA are two known factors contributing to the observed lysozyme resistance of *L. monocytogenes*. Furthermore, it was shown that the absence of components of an ABC transporter, here referred to as EsIABC, leads to a reduction in lysozyme resistance. However, the function of the transporter and how its activity is linked to lysozyme resistance are still unknown. To investigate this further, a strain deleted for the membrane component of the ABC transporter EsIB was constructed. The *esIB* mutant showed a 40-fold reduction in the MIC for lysozyme, confirming previous results. Interestingly, the absence of EsIB had no impact on the resistance towards other cell wall-acting antibiotics, such as penicillin and moenomycin under standard growth conditions. To determine whether the lysozyme sensitivity is a result of a change in the PG structure, the PG was isolated from the wildtype strain 10403S, the *esIB* deletion and an *esIB* complementation strain and analysed by HPLC. The PG of the *esIB* deletion strain is slightly more crosslinked and de-

acetylated as compared to the PG of the wildtype strain. However, it is not clear whether these changes could explain the decreased lysozyme resistance as deacetylation of PG is a modification that supports resistance. The ABC transporter EslABC is transcribed in an operon with a RpiR-regulator. All characterized RpiR regulators are involved in the control of sugar phosphate metabolic pathways. Growth comparisons using different media revealed a growth defect of the *eslB* mutant in the presence of high concentrations of sugars as compared to the wildtype strain. In addition, we observed that the autolysis of the *eslB* deletion strain was drastically increased after growth in BHI medium supplemented with 0.5 M sucrose. The cell lysis of the *eslB* deletion strain could be further enhanced by the addition of penicillin. These findings suggest that the absence of EslB leads to a change in cell integrity of *L. monocytogenes*.

### 083-MCBP

Growth rate constraints in *Corynebacterium glutamicum*

\*S. Matamouros<sup>1</sup>, T. Gensch<sup>2</sup>, I. Abdollahzadeh<sup>2</sup>, J. Hendriks<sup>2</sup>, C. Sachs<sup>1</sup>, N. Tenhaef<sup>1</sup>, M. Graf<sup>3</sup>, R. Takors<sup>3</sup>, S. Noack<sup>1</sup>, K. Nöh<sup>1</sup>, M. Bott<sup>1</sup>

<sup>1</sup>Forschungszentrum Juelich, IBG-1, Juelich, Germany

<sup>2</sup>Forschungszentrum Juelich, ICS-4, Juelich, Germany

<sup>3</sup>University of Stuttgart, IBVT, Stuttgart, Germany

### Introduction

Bacterial growth rate is directly linked to the translational capacity of the cell, which is determined by nutrient availability and ribosome abundance. Ribosomes are large multimeric RNA-protein complexes that constitute a significant fraction of the cellular RNA and protein content. Therefore, ribosome abundance must be tightly controlled to avoid loss of fitness due to resource misallocation. This complex regulation has been mostly studied in *Escherichia coli*, a fast-growing bacterium that exhibits a broad range of growth rates (up to 2.14 h<sup>-1</sup>). *Corynebacterium glutamicum* is an important model organism in industrial biotechnology that although robust and easy to use presents a maximal growth rate (up to 0.6 h<sup>-1</sup>) that lags far behind that for *E. coli*.

### Objective

To understand growth rate constraints in *C. glutamicum* the relationship between growth rate, ribosome abundance and translation elongation rate was examined.

### Methods

Ribosome abundance at different growth rates was determined via super-resolution microscopy using fluorescently tagged ribosomal proteins as well as total RNA-protein ratio. Translation elongation rate at different growth rates was measured via an inducible fluorescent reporter fusion assay.

### Results

The two independent methods for estimating ribosome abundance show that at low growth rates *C. glutamicum* maintains a minimal ribosome level. Ribosome abundance only starts to increase sharply above a certain growth rate, which is approximately the point at which translation elongation rate reaches a maximum.

### Conclusion

*C. glutamicum* maintains a relatively high abundance of ribosomes at low growth rates. As growth conditions improve translation elongation rate increases. Once translation elongation rate reaches its maximum value, growth rate rises via increased ribosome abundance to support the higher demand for protein synthesis. When compared to *E. coli*, *C. glutamicum* produces more ribosomes per cell to support a similar growth rate. This extra burden could be one of the reasons behind the lower maximal growth rate observed for *C. glutamicum*.

### 084-MCBP

Loss of RNase Y provokes massive decrease in transcription

\*M. Benda<sup>1</sup>, L. Krásný<sup>2</sup>, J. Stülke<sup>1</sup>

<sup>1</sup>Institute of Microbiology and Genetics, Georg-August University Göttingen, General Microbiology, Göttingen, Germany

<sup>2</sup>Institute of Microbiology, Czech Academy of Sciences, Laboratory of Microbial Genetics and Gene Expression, Prague, Czech Republic

RNA turnover is an essential regulatory process in all domains of life. The membrane bound endonuclease RNase Y (*rny*) is one of the key components involved in RNA metabolism of the model organism *Bacillus subtilis*. It has been a matter of discussion, whether RNase Y is essential or not, since deletion of *rny* gene is possible, but leads to severe phenotypic effects including defects in cell wall biosynthesis, loss of sporulation, loss of natural competence or reduced growth rate [1]. Furthermore, the *rny* deletion strain is genetically highly unstable and quickly evolves suppressor mutations to at least partially alleviate these defects. To better understand the key role of RNase Y in *B. subtilis* physiology, we have examined several suppressor mutants by whole genome sequencing. This identified the presence of an identical duplication of an about 60 kb long genomic region in all sequenced suppressors, always accompanied by distinct mutations in diverse transcription related genes. The duplicated region is flanked by rRNA operons and contains 76 genes including those coding for all three core subunits of the RNA polymerase –  $\alpha$ ,  $\beta$ ,  $\beta'$ . We hypothesized that duplication of the RNA polymerase genes is the key for this suppression, and therefore decided to interfere with the possibility of a simultaneous duplication of the three genes. For this purpose, the *rpoA* gene (coding for  $\alpha$  subunit) was relocated in the *B. subtilis* genome. Indeed, when the *rny* gene was then deleted in this background and suppressor mutants evolved, no duplication was observed. Instead, each mutant carried a single distinct point mutation in evolutionary conserved regions of genes coding either for the  $\beta$  or  $\beta'$  subunits of the RNA polymerase. *In vitro* transcription assays with the mutated polymerase variants showed massive decrease in transcription efficiency as well as malfunction of common regulatory processes. Altogether, the results suggest a tight connection between RNase Y and the RNA polymerase in maintaining optimal RNA homeostasis within *B. subtilis* cells.

[1] Figaro *et al. J. Bacteriol.* **195** (2013), 2340–2348.

### 085-MCBP

Analysis of putative STRIPAK-interaction partners in

*Sordaria macrospora*

\*A. Gibrón<sup>1</sup>, E. Reschka<sup>1</sup>, S. Pöggeler<sup>1</sup>

<sup>1</sup>Institute of Microbiology and Genetics, Department of Genetics of Eukaryotic Microorganisms, Goettingen, Germany

The striatin-interacting phosphatase and kinase (STRIPAK) complex is a multiprotein complex that is involved in diverse signaling pathways like cell growth and –fusion, cytokinesis,

endocytosis and apoptosis. In animals and fungi, STRIPAK complexes are tightly regulated by striatin-like scaffolding proteins. In the coprophilic ascomycete *Sordaria macrospora* (Sm) the SmSTRIPAK was shown to play an important role in vegetative growth, hyphal fusion and sexual development involving the human striatin homolog PRO11. In addition, the SmSTRIPAK complex consists of the STRIP1/2 homolog PRO22, the SLMAP homolog PRO45, SmMOB3, the scaffolding subunit of phosphatase PP2A SmPP2AA and its catalytic subunit SmPP2Ac1. Recently the coiled-coil protein SmSCI1 was identified as a core subunit of the SmSTRIPAK that interacts with PRO11 and PRO45. In a previous pulldown using SmSCI1-EGFP as bait, the nucleoporin SmPOM33 and the vacuolar protein SmVAC14 were ascertained as putative interaction partners. Moreover, the dynactin component SmARP1 was found in a pulldown with PRO11-EGFP.

Our work focusses on the question in which pathways the SmSTRIPAK might be involved. Here, we analyze the localization of the SmSTRIPAK complex to the nucleus focusing on the identified nucleoporin SmPOM33. We could show the localization of SmPOM33 at the nuclear envelope (NE) and also the endoplasmic reticulum (ER). To characterize SmPOM33, a knockout strain  $\Delta$ Smpom33 was generated for phenotypic analysis. To investigate if the SmSTRIPAK is somehow involved in vacuolar trafficking, the SmVAC14 will be analyzed including the construction of the deletion strain  $\Delta$ Smvac14 for complementation analysis. To learn about the SmSTRIPAK being involved in vesicular transport, the actin-related dynactin compound SmARP1 was investigated and shown to localize at hyphal tips. For further information about the protein, a  $\Delta$ Smarp1 strain will be generated. Furthermore, pulldown-experiments with SmPOM33, SmVAC14 or SmARP1 as bait will be performed to verify the interaction with SmSTRIPAK-complex components.

## 086-MCBP

Host cell signal triggering virulence mechanism in *Escherichia coli*

\*M. M. Khan<sup>1</sup>, K. Sidorczuk<sup>2</sup>, J. Schiebel<sup>1</sup>, S. Guenther<sup>3</sup>, K. Schauler<sup>3</sup>, P. Schierack<sup>1</sup>, R. Kolenda<sup>1,4</sup>

<sup>1</sup>Institute for Biotechnology, BTU Cottbus-Senftenberg, Department of Multiparametric Diagnostics, Senftenberg, Germany

<sup>2</sup>Faculty of Biotechnology, University of Wrocław, Wrocław, Poland

<sup>3</sup>Institute of Pharmacy, Universität Greifswald, Pharmaceutical Biology, Greifswald, Germany

<sup>4</sup>Wrocław University of Environmental and Life Sciences, Wrocław, Poland

### 1. Introduction

*Escherichia coli* colonization and infection results in formation of adhesion patterns which in turn contribute to pathotyping of intestinal *E. coli*. The adhesion patterns of 282 *E. coli* isolates on different cell lines were determined to establish various adhesion patterns such as diffusely distributed, microcolonies, chains and clumps. Only two strains of *E. coli* were found to form huge biofilm like three-dimensional clumpy structures upon adhesion specifically to 5637 cell line.

### 2. Objectives

The objectives of this study are to identify factors determining the tissue host specificity of clumpy adhesion phenotype of *E. coli*.

### 3. Materials & Methods

Genomes of clumpy-forming *E. coli* were sequenced and compared with genome of *E. coli* K-12. RNA was isolated from bacteria forming clumps and bacteria from supernatants during adhesion assays to 5637, PK-15 and Caco-2 cells and sequencing was performed on 100 bp paired-end read Illumina HiSeq2000. Differentially expressed genes were identified with use of edgeR.

### 4. Results

Genome comparison revealed differences between K-12 and clumpy *E. coli*. RNA-Seq data analysis of strain 4972 revealed differential expression of 623 genes between bacteria in clumps and supernatant. Out these 623 genes, 148 genes were unknown or hypothetical proteins. 478 known genes of total 623 were assigned functions in the light previously published research.

It was noted that 36 of total 46 motility and fimbriae genes were downregulated. Interestingly, 87 genes were found to be biofilm associated and 51 of these were upregulated. Stress genes also followed a similar pattern where 69 of 93 genes were upregulated. Only 5 genes were assigned the category of Virulence genes and all of them were slightly upregulated. The most upregulated was Spy periplasmic chaperone protein.

Inactivation of genes in strain 4972 is still being carried out and the initial results show involvement of various genes contributing to clump formation.

### 5. Conclusion

We were able to determine the set of microbial genes that were responsible for clumpy adhesion phenotype upon signal from specific host cells. To confirm role of selected genes deletion mutants will be generated.

## 087-MCBP

The functional diversity of the prokaryotic sulfur carrier protein TusA

\*T. S. Tanabe<sup>1</sup>, S. Leimkühler<sup>2</sup>, C. Dahl<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Mikrobiologie und Biotechnologie, Bonn, Germany

<sup>2</sup>Universität Potsdam, Institut für Biochemie und Biologie, Potsdam, Germany

### Question

The TusA protein has been identified as a central element supplying and transferring sulfur as persulfide to a number of important biosynthetic pathways. In recent years, surprising links between these biosynthetic pathways and prokaryotic metabolism of inorganic sulfur compounds became apparent when the enzymatic generation of persulfidic sulfur as well as sulfur transfer reactions were not only identified as important steps during sulfide detoxification but also recognized as essential components of sulfur oxidation pathways in lithotrophic bacteria and archaea [1]. We intend to categorize the multiple roles of TusA-like proteins in sulfur transfer pathways in different organisms to shed light on the remaining mysteries of this versatile protein.

### Results

Here, we identify common features of all TusA proteins. These include a strictly conserved sulfur-binding cysteine residue within a C-P-X-P motif and  $\alpha\beta\alpha\beta\beta$  fold. In addition,

we summarize predicted and proven roles of TusA proteins. A phylogenetic tree is presented based on 101 proteins representing diverse physiological contexts and genetic environments. This tree does not mirror the phylogenetic tree of prokaryotes. Rather, TusA proteins cluster according to probable cellular functions. This is best exemplified by the TusA proteins related to oxidation of sulfanesulfur in the cytoplasm of dissimilatory sulfur-oxidizing bacteria. They all cluster together although they stem from different bacterial phyla, i.e. the Proteobacteria, the Chlorobi and the Aquificae. Some organisms contain several TusA variants, that have evolved to pursue specific roles in cellular pathways and cannot replace each other. Other organisms like *Allochromatium vinosum* contain only a single copy, which is indispensable for viability.

## Conclusions

TusA has evolved as an optimal and versatile sulfur carrier protein involved in multiple cellular pathways with specific roles adapted to the requirements of each organism. Numerous open questions exist including how target and route specificity is achieved and how pleiotropic cellular defects are caused by the absence of TusA.

[1] Tanabe *et al.* 2019 *Adv Microb Physiol* 75, 233

## 088-MCBP

Protein-protein interactions of the energy generation supracomplex of *Pseudomonas aeruginosa*

\*K. Müller<sup>1</sup>, J. Wissing<sup>2</sup>, L. Jänsch<sup>2</sup>, M. Jahn<sup>1</sup>, D. Jahn<sup>1</sup>

<sup>1</sup>TU Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany

<sup>2</sup>Helmholtz-Zentrum für Infektionsforschung, Zelluläre Proteomforschung, Braunschweig, Germany

Biological energy generation is usually accomplished by membrane spanning electron transport chains composed of multiple protein complexes. Previously, we elucidated involved protein-protein interactions for the denitrification machinery of *Pseudomonas aeruginosa* using a proteomics based interactomics approach (1). A highly complex protein network was observed for the investigated supracomplex responsible for anaerobic energy recovery. Here, we were investigating the dynamics of the corresponding protein complexes for aerobic respiration. For the used interactomics approach 3 bait proteins were selected: NuoJ of the NADH dehydrogenase (complex I), FdhE from electron donating respiratory formate dehydrogenase and NapA as part of the periplasmic nitrate reductase. Tagged versions of the bait proteins were expressed in the corresponding mutant backgrounds. After cultivation at the end of the exponential phase, formaldehyde-mediated protein complex cross-linking was performed. Stabilized protein complexes were purified using Streptavidin-tagged column chromatography and the bound prey proteins then identified via LC/MS/MS. Obtained data were used to reconstruct the protein network of the supracomplex involved in aerobic respiration. Furthermore the same approach was performed with a shift from aerobic to anaerobic growth to investigate the dynamic rearrangements of these complexes.

1) Borrero-de Acuña JM, *et al.* 2016. Protein Network of the *Pseudomonas aeruginosa* Denitrification Apparatus. *J Bacteriol* 198:1401– 1413

## 089-MCBP

Microscopy-based identification and initial characterization of novel inhibitors of bacterial cell division

\*C. Matos de Opitz<sup>1</sup>, P. Sass<sup>1,2</sup>

<sup>1</sup>University of Tuebingen, Microbial Bioactive Compounds, Tübingen, Germany

<sup>2</sup>German Center for Infection Research (DZIF), Tuebingen, Germany

The discovery and implementation of antibiotics as therapeutic agents helped to significantly reduce mortality related to microbial infections. However, the current rise and spread of multi-resistant pathogens, along with a dwindling pipeline in antimicrobial discovery, seriously limit our available options to treat life-threatening bacterial infections and seriously challenges our healthcare systems. Hence, the search for novel antibiotics with promising modes of action is crucial. In this context, we investigate bacterial cell division, an essential process in most bacteria, to explore its potential as a new antimicrobial target pathway. To date, bacterial cell division is not targeted by any clinically applied antibiotic. Antimicrobial compounds affecting cell division in bacteria, often by deregulating the core cell division protein FtsZ, commonly induce a filamentous phenotype in rod-shaped bacteria (1-3). In this study, we used a library of purified natural products from diverse *Streptomyces* species in cell-based assays to identify compounds that provoke such a filamentation phenotype. To this end, we established a semi-automated microscopy method that led to the identification of two novel compounds interfering with bacterial cell division. Current localization studies using cell division proteins as well as fluorescent staining of cellular structures will help to reveal the distinct stages during cell division that are deregulated by these compounds.

1) Sass and Brötz-Oesterhelt. 2013. *Curr Opin Microbiol*, 16, 522-530.

2) Sass *et al.* 2011. *Proc Natl Acad Sci USA*, 108, 17474.

3) Mayer *et al.* 2019. *Int J Med Microbiol*, 151329.

## 090-MCBP

Functional impact of lipid logistics on mycobacterial infection in *Dictyostelium*

\*C. Barisch<sup>1</sup>, I. Hube<sup>1</sup>, S. Deise<sup>1</sup>

<sup>1</sup>University of Osnabrück, Molecular Infection Biology, Osnabrück, Germany

Tuberculosis (Tb) is a wide-spread infectious disease caused by the bacterium *Mycobacterium tuberculosis* (*Mtb*). The high lipid content of this pathogen accounts for many of its unique clinical manifestations. One of the main characteristics of Tb is the formation of lipid-loaded, foamy macrophages during chronic infection. A growing body of evidence indicates that *Mtb* mobilizes lipid droplets (LDs) to scavenge lipids from their host cell. However, how this pathogen remodels the lipid metabolic network of the host is so far poorly understood. Using *Dictyostelium* as experimental model for foamy macrophages in *Mtb* infections, I found that mycobacteria accesses host LDs to build up their own lipid storage organelles and exploits ER-derived phospholipids when LDs are lacking.

Moreover, I observed that mycobacteria that escaped from the mycobacterium-containing vacuole (MCV) to the cytosol recruit LD-derived enzymes and regulatory proteins on their hydrophobic surface. Previous work indicated that these pathogens not only hijack LDs, but also lipid metabolic enzymes and various components of the lipid trafficking machinery.

However, a comprehensive understanding of the underlying molecular principles and their relevance for mycobacterial



infection is missing at present. We will unravel the molecular mechanisms by which mycobacteria acquire and exploit host lipids using the *Dictyostelium/Mycobacterium marinum* infection model. To this end, we will chart the lipid flows between mycobacteria and their host that are potentially relevant for infection and identify lipid species acquired by intracellular mycobacteria using metabolic tracing studies and mass spectrometry lipidomics. In addition, we plan to disrupt lipid flows from the host to the pathogen during infection using genetics or drugs. With the help of fluorescent and clickable lipid probes, we will first analyze the impact of these disruptions on host-to-pathogen lipid flows. Next, we will determine the consequences of blocking specific lipid supply routes on various stages of the mycobacterial infection course. Collectively, these efforts may uncover novel therapeutic targets to fight mycobacteria infection.

---

## 091-MCBP

Filling the gaps:

### Roles of glyceraldehyde 3-phosphate dehydrogenase homologs in *Pseudomonas aeruginosa* metabolic fluxes

\*A. Klotz<sup>1</sup>, U. Jenal<sup>1</sup>

<sup>1</sup>University of Basel, Biozentrum, Basel, Switzerland

*Pseudomonads* are ubiquitous, environmental bacteria with a great potential for rapid adaptation to changing environmental conditions. A highly versatile and tightly regulated metabolism allows *Pseudomonas aeruginosa* to adapt to various environmental conditions, infect a wide range of hosts, and successfully dwell on different host tissues causing acute and chronic infections. Thus, *P. aeruginosa* is one of the most dangerous human pathogens and has recently been listed as one of the highest priority infectious threats by the World Health Organization<sup>1</sup>. Surprisingly, little is known about the role of the central carbon metabolism and its complex regulation within the host.

Here, we analysed distinct physiological functions of three paralogs of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), a key enzyme of the central carbon metabolism. Preliminary experiments indicated that while GapA is bi-functional, GapB and GapC primarily catalyse glycolytic and gluconeogenic reactions, respectively. To dissect the role of the single GAPDH, transcriptional reporters were constructed allowing us to examine the carbon fluxes *in vivo* and at single cell level, notifying distinct expression profiles depending on growth phases and medium composition, used for growth. In contrast to *gapA* and *gapB* mutants, a strain lacking GapC completely lost its virulence in a *Galleria mellonella* animal model, indicating that in this host gluconeogenesis is of key importance for the pathogen.

This study contributes to a better understanding of how carbon metabolism contributes to growth of this important human pathogen providing detailed insights into the control of central metabolic processes. The long-term goal is to use this information to assess important bacterial metabolic processes in the human patient.

<sup>1</sup> Tacconelli, E. et al. Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. The Lancet Infectious Diseases 18, 318–327 (2017).

---

## 092-MCBP

Discovery and verification of cellular substrates of the ClpXP protease involved in bacteriochlorophyll *a* biosynthesis in *Dinoroseobacter shibae*

\*A. Rommerskirch<sup>1</sup>, D. Jahn<sup>1</sup>, E. Härtig<sup>1</sup>

<sup>1</sup>Technische Universität Braunschweig, Institute of Microbiology, Braunschweig, Germany

**Introduction:** The marine bacterium *Dinoroseobacter shibae* is capable to perform aerobic anoxygenic photosynthesis. Screening of our transposon mutant library of *D. shibae* for loss of pigmentation and Bchl *a* absorbance identified Dshi\_1387, encoding the ATP-dependent protease subunit ClpX of the ClpXP protease in *D. shibae*. Transcriptome analyses comparing transcript levels of the photosynthetic gene cluster (PGC) from wild type strain *D. shibae* DFL12T with  $\Delta clpX$  mutant strain grown in the dark indicated a light-dependent regulation of photosynthetic genes.

**Objectives:** Determination of cellular substrates of the ClpXP protease involved in Bchl *a* biosynthesis and pigmentation in *D. shibae*.

**Material & Methods:** To identify substrates of the ClpXP protease, a proteolytic inactive form of ClpP (ClpP<sup>trap</sup>) was expressed in the *D. shibae*  $\Delta clpP$  mutant strain that will retain but not degrade substrates translocated into its proteolytic chamber. Substrates captured inside the proteolytic barrel were co-purified along with the Strep-tagged ClpP complex and identified by LC-MS/MS. To verify potential substrates *in vitro* and *in vivo* degradation assays were established. Potential substrates were heterologously produced in *E. coli* as Strep-tagged fusionproteins and subsequently exposed to an active ClpXP protease system *in vitro*. For the *in vivo* protease assay, the potential targets were expressed homologously in *D. shibae*. Degradation of the expressed target proteins will be monitored by Western Blot analysis.

**Results:** About 78 potential substrates of the ClpXP protease were identified in *D. shibae*. Interactionpartners of ClpP like ClpX or ClpA were co-purified. Moreover, proteins already known to be ClpXP targets like the transcriptional regulators CtrA and LexA were identified indicating the validity of the established trapping system. Newly identified ClpXP substrates include proteins essential for bacteriochlorophyll *a* biosynthesis: 5- aminolevulinate synthase (HemA1, HemA2) and subunit I and H of the magnesium chelatase (Bchl, BchH).

**Conclusion:** Our study support the role of proteolysis by ClpXP for the bacteriochlorophyll *a* biosynthesis pathway in *D. shibae*.

---

## 093-MCBP

### PHB in cyanobacteria is not what it is meant to be

\*M. Koch<sup>1</sup>, K. Forchhammer<sup>1</sup>

<sup>1</sup>University Tübingen, Organismic Interaction, Tübingen, Germany

Poly-Hydroxy-Butyrate (PHB) is a very versatile biopolymer and present in numerous microbial species. Among its functions are the protection against UV light, oxidative pressure, osmoregulation and many others. However, its main function is to serve as a storage polymer.

It is known for more than 60 years, that PHB is also produced in cyanobacteria. A recent study found its

presence in 134 out of 137 investigated cyanobacterial species (Kaewbai-Ngam et al., 2016). However, its physiological function for this phylum of bacteria remains unknown.

The aim of this study was to gain further insights into the importance of PHB for a cyanobacterial lifestyle. Besides at growth under nutrient depleted conditions, such as nitrogen or phosphorus limitation, PHB related genes were also upregulated during the night. We compared viability between *Synechocystis* sp. PCC 6803 wildtype and a PHB-free mutant ( $\Delta$ PhaEC) under numerous abiotic stress-conditions and analyzed the recovery from prolonged nutrient starvation. However, in none of the tested conditions the PHB-deficient mutant showed reduced fitness. Hence, the true physiological function of PHB in cyanobacteria remains puzzling.

KAEWBAI-NGAM, A., INCHAROENSAKDI, A. & MONSHUPANEE, T. 2016. Increased accumulation of polyhydroxybutyrate in divergent cyanobacteria under nutrient-deprived photoautotrophy: An efficient conversion of solar energy and carbon dioxide to polyhydroxybutyrate by *Calothrix scytonemicola* TISTR 8095. *Bioresour Technol*, 212, 342-347.

---

#### 094-MCBP

Analysis of STRIPAK-dependent phospholipid signaling in *Sordaria macrospora*

\*S. Erb<sup>1</sup>, E. Reschka<sup>1</sup>, S. Pöggeler<sup>1</sup>

<sup>1</sup>Institute for Microbiology and Genetics, Department for Genetics of Eukaryotic Microorganisms, Göttingen, Germany

Multicellular development and differentiation in eukaryotes needs tightly controlled signaling mechanisms. One multi protein complex involved in this process of regulation is the striatin-interacting phosphatase and kinase (STRIPAK) complex. This complex is conserved in metazoans and fungi and owns especially highly conserved components between fungi and animals. The STRIPAK complex is involved in various signaling processes like cell growth and -fusion, cytokinesis, endocytosis and apoptosis and is tightly regulated by striatin-like scaffolding proteins. In the coprophilic ascomycete *Sordaria macrospora* (Sm), the SmSTRIPAK complex is important for the control of fungal sexual development, hyphal fusion and vegetative growth. The SmSTRIPAK complex consists of the human striatin-like homologue PRO11, the STRIP1/2 homologue PRO22, the SLMAP homologue PRO45, SmMOB3 and the scaffolding subunit of phosphatase PP2A, named SmPP2AA, including its catalytic subunit SmPP2Ac1. Additionally, recent studies identified another core subunit of the SmSTRIPAK complex: the coiled-coil protein SmSCI1, which interacts with PRO11 and PRO45. LCMS analysis of SCI1 and PRO11 pull downs revealed an interaction with an orthologue of the yeast PDR16 phosphatidylinositol transfer protein and a putative interaction with the oxysterol-binding protein OSH6, indicating a potential role of the SmSTRIPAK in phospholipid signaling.

The interaction of SmSTRIPAK components with PDR16 and OSH6 will be verified by Co-IP experiments and co-localization studies. The ongoing generation of the deletion strains  $\Delta$ Smpdr16 and  $\Delta$ Smosh6 will be accomplished to analyze their functions with regard to fruiting-body development and hyphal fusion. To monitor dynamic changes in the patterns of phospholipids with regard to the STRIPAK complex, fluorescent biosensors, consisting of

lipid-binding domains that specifically interact with known phosphatidylinositol phosphate or phosphatidylserine species, will be analyzed in wild type (wt) and STRIPAK mutant strains. Additionally, changes in phospholipid composition of membranes will be analyzed of samples from  $\Delta$ pdr16 and  $\Delta$ osh6 in comparison to the wt.

---

#### 095-MCBP

Transcriptional organization of magnetosome gene cluster in *Magnetospirillum gryphiswaldense*

\*C. N. Riese<sup>1</sup>, D. Schüler<sup>1</sup>

<sup>1</sup>University of Bayreuth, Department of Microbiology, Bayreuth, Germany

The Alphaproteobacterium *Magnetospirillum gryphiswaldense* orients itself along the earth's magnetic field by biomineralizing membrane enveloped magnetite crystals, the magnetosomes. The approximately 30 genes with diverse functions in iron uptake, membrane vesicle formation, magnetite biomineralization and magnetosome chain formation are organized in the operons *mamAB* (16 kb), *mamGFDC* (2 kb), *mamXY* (5 kb), *mms6op* (3.5 kb) and *feoAB* (2.5 kb), which are clustered in a single chromosomal region called Magnetosome Island (MAI) 1.

However, transcriptional regulation as well as promoters are largely unknown. To address these questions, we applied up-to-date transcriptome-wide techniques to investigate the transcriptional organization as well as differential expression under various well-defined oxygen conditions for magnetosome biosynthesis in an oxystat-fermenter.

Preliminary data reveal a complex transcriptional architecture with multiple transcriptional start sites (TSS), regulatory and terminator regions. In case of *mamAB* two additional TSS within coding regions of *mamH* and *mamQ* could be detected, indicating transcription as at least two sub-operons. Additionally, novel termination and putative regulator sites could be detected by Term-sequencing, further expanding the transcriptional architecture of the *mam*-operons. Furthermore, oxygen conditions affect transcriptional expression of several *mam*-operons for example *mms6op* showing significant downregulation under oxic conditions in comparison to microoxic standard conditions. Moreover, organization of *mamGFDC* and *feoAB* in single operons with one primary promoter and distinct transcriptional termination could be verified.

The gathered insights uncover a much more complex transcriptional organization as previously assumed, expanding the current transcriptional model by a new level of *mam*-gene regulation. In depth-bioinformatic mining of the datasets for conserved promoter- as well as regulatory sequence motives is in progress to further enhance knowledge about magnetosome bioproduction regulatory networks.

1 Uebe R. and Schüler D. (2016) *Nat. Rev. Microbiol.* 14:621–637.

---

#### 096-MCBP

The author has not agreed to a publication.

---

#### 097-MCBP

Surface Acclimation of the Cyanobacterium *Synechocystis* sp. PCC 6803

Many bacteria exhibit planktonic as well as sessile lifestyles. We investigate the acclimation to these environmental conditions in the cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*).

To this end, a microarray experiment of *Synechocystis* cells, which were incubated either on agar plates or in liquid medium, was performed. It revealed, amongst others, an altered transcription of genes potentially involved in extracellular polymeric substances (EPS) production. Indeed, we could determine varying extracellular polysaccharide compositions between sessile and planktonic cells. Both findings hint at a role of EPS in surface acclimation and biofilm formation in cyanobacteria.

Furthermore, transcription level of minor pilin genes differ between the two conditions in the microarray analysis. The *Synechocystis* genome encodes at least 10 potential minor pilin proteins with so far unknown functions. In *Pseudomonas aeruginosa*, a role of the minor pilins in pilus formation and function is proposed. Now, our studies of minor pilin mutants in *Synechocystis* reveal several distinct roles of these proteins. They seem to be involved in attachment to surfaces, flocculation, motility and natural competence.

Upon acclimation to surfaces, levels of second messengers, such as c-di-GMP and cAMP, change rapidly. A microarray analysis demonstrates that the level of the second messenger c-di-GMP alters the minor pilin gene transcription. Moreover, other genes encoding cell surface components, such as chaperone usher pili, were differentially transcribed.

In conclusion, we propose that sensing of surfaces leads to modified levels of the second messengers c-di-GMP and cAMP, resulting in an altered transcription of minor pilin genes. Thus, we assume that minor pilins of *Synechocystis* are involved in surface acclimation.

---

## 098-MCBP

### Cold-shock proteins are key players in the physiology of *Bacillus subtilis*

\*P. Faßhauer<sup>1</sup>, J. Stülke<sup>1</sup>

<sup>1</sup>Institute for Microbiology and Genetics, General Microbiology, Göttingen, Germany

RNA chaperones are RNA binding proteins that aid folding of RNA to their functional secondary structure. They influence cellular processes such as transcription, translation and RNA degradation. RNA chaperones such as Hfq, the ProQ/FinO family, or the cold-shock proteins (CSPs) and their action on specific RNAs have been well studied in *Escherichia coli*. For Hfq as well as the CSPs, homologs exist in *Bacillus subtilis*. CSPs have been shown to bind RNA and ssDNA and have been proposed to act as RNA chaperones. The aim of our study is to uncover their mechanism of action and RNAs affected by them in *B. subtilis*. A *cspB cspD* double mutant has lost competence, exhibits a strong reduction in growth rate and genetic stability indicating the critical importance of these RNA chaperones. We isolated suppressors and identified either a point mutation in the ribosomal binding site (RBS) of the *veg* gene of unknown function, or point mutations in the 5'UTR of *cspC*. The mutation in the RBS of *veg* lowers its expression. The construction of a *cspB cspD veg* triple mutant indicated that *veg* expression prevented growth of the strain lacking the major RNA chaperones.

Promoter-reporter gene fusions showed that the mutation in the 5'UTR of *cspC* results in a two-fold increased *cspC* expression. Promoter activity assays showed that expression of *cspC* is up-regulated at 15°C and down-regulated by CspB and CspD but not CspC. RNA fish experiments with CspD followed by RNA-Sequencing suggested binding of CspD to ribosomal RNAs. Our results demonstrate that the expression of *cspC* is tightly regulated and that the presence of CSPs is essential for the viability of *B. subtilis* even at 37°C. We identified multiple possible targets of the CSP proteins and anticipate our results to be the starting point to uncover their mechanism of action.

---

## 099-MCBP

### Targeting the cell division protein FtsZ by antibiotic action

\*N. Silber<sup>1</sup>, S. Pan<sup>1</sup>, S. Schäkermann<sup>2</sup>, C. Mayer<sup>1</sup>, H. Brötz-Oesterhelt<sup>1</sup>, P. Sass<sup>1</sup>

<sup>1</sup>University of Tübingen, Department of Microbial Bioactive Compounds, Tübingen, Germany

<sup>2</sup>Ruhr University Bochum, Applied Microbiology, Bochum, Germany

ADEP antibiotics act by deregulating ClpP, the proteolytic core of bacterial Clp proteases. By this means, ADEPs effectively kill multidrug-resistant Gram-positive pathogens including enterococci (VRE), streptococci (PRSP), and *Staphylococcus aureus* (MRSA) (1). For regulated proteolysis, ClpP strictly depends on the interaction with partner Clp-ATPases that together with adapter proteins are responsible for substrate recognition as well as for unfolding and feeding the substrates into the barrel-shaped ClpP core (2). ADEPs activate ClpP in the absence of such regulatory Clp-ATPases, conferring independent proteolytic activity to the otherwise dormant proteolytic core (3,4). Although ADEP-mediated activation of ClpP may lead to degradation of nascent polypeptides and other protein substrates in the bacterial cell, not all proteins seem equally susceptible. In a previous study, we identified the cell division protein FtsZ to be particularly sensitive to ADEP-ClpP (5), and to date FtsZ is the only folded bacterial protein that has been confirmed to be degraded by ADEP-activated ClpP *in vitro* as well as in living cells. By analyzing a series of FtsZ mutant proteins using biochemical assays, we unraveled the molecular reasons for this preferred degradation of FtsZ. Unexpectedly, ADEP-ClpP leads to the unfolding of the fully-folded globular core region of FtsZ in the absence of any ATP-fueled Clp-ATPase, thereby providing insight into the structural flexibility of the FtsZ protein. Importantly, such an intrinsic unfolding capability of ClpP has yet not been reported. Furthermore, FtsZ degradation is hampered by nucleotide binding. Using super-resolution fluorescence time-lapse microscopy, we thus followed the fate of FtsZ rings in ADEP-treated bacteria, which provided new insight into the activity of ADEP inside the bacterial cell.

- 1) Brötz *et al.* 2005, Nat Med 11: 1082-87
- 2) Kirstein *et al.* 2009, Nat Rev Microbiol, 7:589-599
- 3) Kirstein *et al.* 2009, EMBO Mol Med. 1: 37-49
- 4) Gersch *et al.* 2015, Nat Commun, 6: 6320
- 5) Sass *et al.* 2011, Proc Natl Acad Sci USA, 108(42):17474-17479

---

## 100-MCBP

### The role of protein secretion systems in prey cell killing of the predatory soil bacterium *Myxococcus xanthus*

\*S. Thiery<sup>1</sup>, C. Kaimer<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Department for Biology and Biotechnology, Bochum, Germany

*Myxococcus xanthus* is a social soil bacterium that exhibits distinct multicellular behavior. Moreover, *M. xanthus* is a predator that actively kills a broad range of bacterial species to feed on the released biomass. It has been established that predation by myxobacteria is a complex, multi-component process that involves hydrolytic enzymes, secondary metabolites and outer membrane vesicles. However, the detailed molecular mechanism of prey cell killing remains largely unknown. Real-time microscopy of *M. xanthus* predation towards *Escherichia coli* on single cell level revealed that lysis of prey occurs within several minutes and only upon direct cell-cell contact. This observation suggests that *M. xanthus* secretes lytic factors in a targeted manner and possibly dedicated protein secretion systems.

To investigate the putative role of protein secretion systems for the predation process, we generated deletion mutants and analyzed their predatory behavior. Our analysis includes co-cultivation assays that assess predation of different prey bacteria on population and single-cell level, and on time scales ranging from 30 minutes up to several days. Deletion of the *M. xanthus* type VI secretion system did not significantly affect predation of Gram-negative and Gram-positive prey bacteria. However, a mutation in an alternative type II secretion system resulted in a predation defect on Gram-negative prey, while predation of Gram-positive bacteria was unaffected. This distinct predation phenotype allows us to further exploit secretome differences between the *M. xanthus* mutant and wild type strain, potentially leading us to the identification of unknown protein effectors that kill prey bacteria.

#### 101-MCBP

AmyGreen is a new amyloid-sensitive stain for biofilm research

\*O. Moshynets<sup>1</sup>, S. Chernii<sup>1</sup>, Y. Snihirova<sup>1</sup>, R. Czerwieniec<sup>2</sup>, V. Chernii<sup>3</sup>, S. Yarmoluk<sup>1</sup>, V. Kovalska<sup>1</sup>

<sup>1</sup>*Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, Kiev, Ukraine*

<sup>2</sup>*Regensburg University, Regensburg, Germany*

<sup>3</sup>*Institute of General and Inorganic Chemistry NASU, Kiev, Ukraine*

Pathological amyloid fibrils are high stable protein aggregates that are associated with some human conformational diseases. The functional amyloids appeared in bacterial biofilms where they may enhance bacterial virulence and pathogens spread. Since fluorescence assay is an effective tool for detection of fibrillar aggregates, there is an interest in the development of new amyloid-specific dyes for their visualization.

We aimed to study the amyloid sensitivity and specificity of the new dye AmyGreen.

Insulin and lysozyme were used to create amyloid fibrils in vitro. CLSM was used to visualize amyloids in biofilms.

Here we report that alkylamino  $\beta$ -ketoenole dye AmyGreen is able to detect both amyloid-produced biofilms and pathological fibrils. Beta-ketoenole dyes were shown as dyes able to increase the emission intensity in the presence of insulin and lysozyme fibrils with quantum yield up to 0.5. AmyGreen possesses no fluorescent response upon presence of dsDNA and RNA, while other widely used amyloid-sensitive dye Thioflavin T shows fluorescent response on RNA presence (quantum yield reaches 0.14). CLSM was used to assess an applicability of AmyGreen as a probe for fluorescence imaging of amyloid fibrils in bacteria biofilms. It was shown that AmyGreen visualized matrix components in biofilms of amyloid-produced bacterial strains

*E. coli* K12, *S. aureus* ATCC25923, *P. fluorescens* SBW25, *K. pneumoniae* ATCC 27853; *B. avium* 61. AmyGreen allowed visualization of structural elements of the biofilm matrix with a higher resolution compared to Thioflavin T. Co-staining of bacteria biofilm *E. coli* K12 by AmyGreen with an EtBr allowed suggesting that the dye did not bind to nuclear and extracellular DNA such as stain Thioflavin T did. Co-staining of AmyGreen and Calcofluor-white of cellulose-containing *E. coli* K12 biofilm demonstrates a difference in staining patterns of stains, which suggests no high specific binding of beta-ketoenole to biofilm matrix polysaccharides. Observed dyes co-localization is probably connected with the location of bacteria on cellulose islands.

Thereby, we proposed AmyGreen as a stain for visualization of amyloids in bacterial biofilms.

#### 102-MCBP

##### Demonstration of a novel PLP-dependent decarboxylation-oxygenation of pyoverdine as catalyzed by PvdN from *Pseudomonas fluorescens*

\*M. F. Sugue<sup>1</sup>, A. Burdur<sup>1</sup>, G. Dräger<sup>2</sup>, T. Brüser<sup>1</sup>

<sup>1</sup>*Leibniz Universität Hannover, Institut für Mikrobiologie, Hannover, Germany*

<sup>2</sup>*Leibniz University Hannover, Institute of Organic Chemistry, Hannover*

Fluorescent pseudomonads are wide-spread bacteria, including well-known pathogens, such as *Pseudomonas aeruginosa*, or important plant growth promoting rhizobacteria, such as *Pseudomonas fluorescens*. Under the iron-limited conditions typically found in host environments, these pseudomonads produce fluorescent siderophores, termed pyoverdines, from N-terminally acylated non-ribosomal peptides that undergo multiple maturation steps after being transported into the periplasm. First, the N-terminal acylation is removed, followed by cyclization and oxidation steps that result in formation of the fluorophore. In addition, a strictly conserved N-terminal glutamate residue is modified. While the deacylation likely permits the release of the pyoverdine and while the fluorophore formation is important for the formation of two of the six ligands for iron complexation, the function of the N-terminal modifications is not known so far. We recently have identified the enzymes that are responsible for the conversions of the N-terminal glutamate. PtaA is a transaminase that converts the glutamate into  $\alpha$ -ketoglutarate [1], whereas PvdN is a novel PLP-dependent enzyme that is required for the transformation of the glutamate to succinamide [2]. The latter reaction has so far only indirectly been concluded from *in vivo* analyses of wild type and *pvdN* mutant strains. Here we report the purification of PvdN and of its substrate, the glutamate variant of the pyoverdine from *Pseudomonas fluorescens* strain A506. With these components, we could demonstrate the direct enzymatic conversion of the glutamate form of pyoverdine to the succinamide form. Thereby, the *in vitro* data confirm that PvdN indeed is able to catalyze the novel PLP-dependent decarboxylation-oxygenation reaction.

[1] Ringel, M.T., Dräger, G., and Brüser, T. (2017) The periplasmic transaminase PtaA of *Pseudomonas fluorescens* converts the glutamic acid residue at the pyoverdine fluorophore to  $\alpha$ -ketoglutaric acid. *J. Biol. Chem.* **292**, 18660-18671

[2] Ringel, M.T., Dräger, G., and Brüser, T. (2016) PvdN enzyme catalyzes a periplasmic pyoverdine modification. *J. Biol. Chem.* **291**, 23929-23938

---

### 103-MCBP

#### Construction of a fluorescence-based reporter gene system for *in vivo* analysis of differential gene expression and subpopulation-analysis in *Staphylococcus aureus*

\*H. Wolfgramm<sup>1</sup>, P. Hildebrandt<sup>1</sup>, S. Michalik<sup>1</sup>, U. Völker<sup>1</sup>, A. Reder<sup>1</sup>

<sup>1</sup>University Medicine Greifswald, Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Germany

*Staphylococcus aureus* is an opportunistic pathogen and causes a multiplicity of important clinical infections. Although *S. aureus* is classically considered as an extracellular pathogen recent studies indicate an association between chronic courses of disease and long-term persistence of internalized bacteria in host cells. The adaptation to intracellular persistence is a dynamic process and seems to enable *S. aureus* to escape the hosts immune system and antibiotic treatments. Persisting intracellular *S. aureus* are characterized by upregulation of SigB and simultaneous downregulation of *agr*. Nevertheless, little is known about gene regulation determining the dynamic decision between growing and non-growing persisting subpopulations. Besides SigB, CodY may play a crucial role by regulating the *agr* and SaeRS system. We have developed a reporter vector to examine up to three different physiological states during internalization and long term infection/colonization. The reporter vector pTricolor contains the three codon optimized fluorescent reporter genes *d-tomato*, *e-gfp* and *e-cfp*. The *d-tomato* gene is constitutively expressed from an optimized *pgl*-SigA promoter and allows copy-number correction and normalization between different experiments. To investigate the activation pattern of SigB and CodY *e-gfp* is placed under the control of the *clpL* promoter and *e-cfp* under the control of the *saHPF* promoter, respectively (both derived from *S. aureus subsp. aureus* NCTC 8325). Reportergene expression is monitored by flow cytometry and fluorescence microscopy. We show that pTricolor is stably passed on also under non-selective conditions. The induction of SigB and CodY regulated promoters was shown under defined *in vitro* conditions. Thus pTricolor is a suitable reporter vector to investigate central questions of differential gene regulation of a pathogen and characterization of subpopulations during *in vivo* infection.

---

### 104-MCBP

Colonizing bacteria increase the pro-inflammatory immune response after secondary influenza virus infection

\*J. Wilden<sup>1</sup>, E. Hrinčius<sup>1</sup>, S. Niemann<sup>2</sup>, S. Ludwig<sup>1</sup>, C. Ehrhardt<sup>1,3</sup>

<sup>1</sup>Westfälische Wilhelms-University, Institute of Virology, Muenster, Germany

<sup>2</sup>Westfälische Wilhelms-University, Institute of Medical Microbiology, Muenster, Germany

<sup>3</sup>Jena University Hospital, Institute of Medical Microbiology, Section of Experimental Virology, Jena, Germany

Influenza viruses (IV) are causative agents of severe respiratory infections. Beyond the virulence of the virus itself, epidemiological data suggest that bacterial superinfections are the major cause for increased morbidity and mortality. Hallmarks of disease are enhanced pathogen load, dysregulation of immune response and tremendous tissue damage. Among the bacteria that induce secondary pneumonia, *Staphylococcus aureus* (*S. aureus*) has been frequently identified as being present during severe influenza seasons. However, half of the population either carries the human commensal *S. aureus* persistently or intermittently without showing any medical symptoms. In consequence we focused the question how primary bacterial exposure affects a secondary IV-infection.

Therefore, we infected A549 human lung epithelial cells for 24 h with the *S. aureus* patient isolate 3878 followed by a secondary IV infection for up to 32 h [A/Puerto Rico/8/34 (H1N1); A/Panama/1/68 (H3N2)]. At different times of infection progeny viral and bacterial titers were analyzed. Pathogen-mediated effects on protein and mRNA expression of cytokines and chemokines were investigated by FACS analysis and qRT-PCR.

Interestingly, the infection with *S. aureus* 3878 prior to IV infection neither resulted in changes of viral and bacterial titers nor affected the expression of specific antiviral genes in comparison to single IV infections. However, in presence of both pathogens, enhanced proinflammatory cytokine and chemokine levels were detected compared to single-infected cells. By use of an 84-gene PCR array we were able to detect changes in the regulation of cellular signaling factors that are involved in the mounting of innate immune responses. Among these NOD-like-, TLR- and RIG-I-mediated signaling pathways seem to be of major importance.

So far our data indicate a minor effect of colonizing bacteria on antiviral responses. Nevertheless, a significant impact of colonizing bacteria on the regulation of pro-inflammatory factors was observed, probably contributing to an imbalance of cell intrinsic innate immune response and severe outcome of infection.

---

### 105-MCBP

#### Characterization of an RNase III mutant strain of *Rhodobacter sphaeroides*

\*J. Börner<sup>1</sup>, G. Klug<sup>1</sup>

<sup>1</sup>Institut für Mikrobiologie und Molekularbiologie, Giessen, Germany

As bacteria are exposed to an ever-changing environment, they have evolutionarily developed complex regulatory networks to adapt their proteome to different environmental conditions. At the same time, protein biosynthesis is one of the most resource- and energy intensive biological processes in all living organisms. Therefore bacteria use different exo- and endoribonucleases to regulate gene expression quickly by degrading transcripts that are no longer needed. RNase III is a highly conserved dsRNA processing endoribonuclease. Previous studies have shown that RNase III plays a crucial role in RNA metabolism, by regulating the abundance of rRNAs, mRNAs and regulatory ncRNAs. In this work an RNase III deletion mutant of the facultative phototrophic bacterium *Rhodobacter sphaeroides* was characterized. The aim of this study was to identify differences in phenotype and RNA physiology between wild type and mutant, which can be explained by a loss of RNase III activity. We analyzed the growth behaviour of the mutant and the wild type under different growth conditions, including oxidative and cadmium stress. As *Rhodobacter sphaeroides* is known for its metabolic versatility, we were also interested in regulation of the transcriptome, especially under oxidative, heat and cadmium stress conditions. To investigate the influence of RNase III on gene expression, we performed Northern blot and qRT-PCR experiments. The results suggest that the deletion of the RNase III coding gene widely impacts phenotype and transcriptome in *Rhodobacter sphaeroides*.

---

### 106-MCBP

#### A-to-I mRNA editing changes protein functions for distinct developmental processes

B. Blank-Landeshammer<sup>1</sup>, H. Strotmeier<sup>2</sup>, R. Lütkenhaus<sup>3</sup>, M. Nowrousian<sup>3</sup>, A. Sickmann<sup>1</sup>, U. Kück<sup>4</sup>, \*I. Teichert<sup>2</sup>

<sup>1</sup>Leibniz-Institut für Analytische Wissenschaften – ISAS e.V., Protein Dynamics Group, Dortmund, Germany

<sup>2</sup>Ruhr University Bochum, General and Molecular Botany, Bochum, Germany

<sup>3</sup>Ruhr-Universität Bochum, Lehrstuhl für Molekulare und Zelluläre Botanik, Bochum, Germany

<sup>4</sup>Ruhr-University Bochum, AK für Allgemeine und Molekulare Botanik, Bochum, Germany

**Introduction:** A-to-I editing of nuclear transcripts has recently been described in fungi, but occurs also in metazoan and in bacteria. In fungi from the class of ascomycetes, editing has hitherto only been detected during the formation of sexual fruiting bodies. Editing sites mostly reside in coding regions, potentially leading to changes in the amino acid sequence of the encoded proteins. Remarkably, editing also targets UAG stop codons, leading to the formation of UGG tryptophan codons and C-terminally elongated proteins (stop loss). However, how fungal editing is catalyzed, which editing sites are of biological relevance and if editing evolved for the purpose of fruiting body formation is still unclear.

**Objectives:** Our study aimed to identify editing sites that cause protein changes, quantify these changes and analyze their biological role during development in the model ascomycete *Sordaria macrospora*.

**Materials and Methods:** We used RNA-seq, Proteomics and Proteogenomics to identify editing sites. Further, we characterized genes whose transcripts undergo editing by generating deletion strains as well as complemented strains carrying different alleles.

**Results:** We identified a diverse set of editing sites in distinct sexual tissues by RNA-seq as well as Proteogenomics. Proteomics and Proteogenomics provided evidence that editing indeed changes protein sequences. Quantitative mass spectrometry showed that C-terminally elongated protein isoforms from stop loss editing accumulate in maturing sexual tissues. We show that transcripts undergoing editing encode proteins required for proper sexual spore formation and that both, short genome-encoded protein isoforms as well as long protein isoforms generated by editing, have functional significance in this process.

**Conclusion:** A-to-I RNA editing in fungi leads to functionally different protein isoforms occurring simultaneously. Since editing is highly correlated with the formation of fruiting bodies, we hypothesize that a diversified proteome is a prerequisite for the formation of generative tissues and sexual spores, a much understudied process that requires massive cellular re-organization.

## 107-MCBP

### ***Bacillus subtilis* mainly copes with the loss of the glutaminase domain PdxT of the pyridoxal synthase complex PdxST by genomic amplification of the synthase domain gene *pdxS***

\*B. Richts<sup>1</sup>, A. Poehlein<sup>2</sup>, R. Daniel<sup>2</sup>, F. M. Commichau<sup>2</sup>

<sup>1</sup>Institute of Microbiology and Genetics, General Microbiology, Göttingen, Germany

<sup>2</sup>Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology, Göttingen, Germany

Pyridoxal-5"-phosphate (PLP), the biologically active form of Vitamin B6, serves as an essential cofactor for roughly 4% of the known enzymatic reactions. Two pathways for *de novo* synthesis of PLP exist in nature: the DXP-dependent pathway, which is a 7-step reaction occurring in  $\alpha$ -/y-proteobacteria and the DXP-independent pathway that is

present in lower eukaryotes, plants and in some bacteria, including the Gram-positive model bacterium *Bacillus subtilis*. *B. subtilis* synthesizes vitamin B6 via the pyridoxal synthase (PdxST) complex, which converts glutamine together with pentoses and trioses to PLP. PdxT is the glutaminase subunit that cleaves glutamine to 2-oxoglutarate and ammonium, of which the latter is provided to the PLP synthase domain PdxS. PdxS can also synthesize PLP in the absence of PdxT if high amounts of ammonium are provided. In this study, we addressed the question of how a *B. subtilis* *pdxT* mutant strain adapts to PLP limitation during growth with low levels of ammonium. We hypothesized that amino acid exchanges might improve the activity of the PLP synthase subunit and thus allow the bacteria to synthesize sufficient PLP for rapid growth under ammonium limitation. For this purpose, we established a reporter system to distinguish between different classes of suppressor mutants. Genome sequencing analyses revealed that all isolated suppressor mutants had amplified a 16 kb-long genomic region containing *pdxS* gene. A subset of the suppressor mutants had acquired mutations in the *tnrA* and *glnA* genes encoding the transcriptional regulator TnrA and the glutamine synthetase GlnA, respectively. TnrA is involved in global nitrogen regulation and GlnA controls the DNA-binding activity of TnrA. The characterization of the suppressor mutants carrying mutations in the *tnrA* and *glnA* genes revealed that the uptake of ammonium by the bacteria was enhanced. Thus, the amplification of the *pdxS* gene and the enhanced ammonium uptake compensate for the loss of the *pdxT* gene. Another subset of suppressor mutants had acquired mutations in genes that are unrelated to nitrogen metabolism. The characterization of these mutants is underway and will be presented.

## 108-MCBP

### **Dissecting the binding behavior of the multi-RRM protein Rrm4 during endosomal mRNA transport in *Ustilago maydis***

\*N. Stoffel<sup>1</sup>, L. Olgeiser<sup>2</sup>, M. Feldbrügge<sup>1,3</sup>

<sup>1</sup>Heinrich-Heine University, Institut für Microbiology, Düsseldorf, Germany

<sup>2</sup>Heinrich-Heine University, Düsseldorf, Germany

<sup>3</sup>Heinrich-Heine University, Institut für Microbiology, Düsseldorf, Germany

Transport of mRNAs is crucial to regulate protein expression at the spatio-temporal level. The localization of mRNA is coupled to the intracellular transport machineries by RNA-binding proteins (RBPs). A well-studied translocation mechanism is the endosomal mRNA transport along microtubules in infectious hyphae of the plant pathogen *Ustilago maydis*. The key component of endosomal mRNA transport is the RBP Rrm4, which is crucial for proper growth of fungal hyphae. Rrm4 contains three RNA Recognition Motifs (RRM) and two MLE for protein-protein interactions. Recently, we obtained a transcriptome-wide view of Rrm4 and an accessory RBP Grp1 by using a comparative *in vivo* UV-crosslinking approach.

Rrm4 binds predominantly to the 3'UTR of target transcripts and landmarks of translation, such as start and stop codons, which, this supports the current model of local translation on endosomes. Furthermore, Rrm4 binds the sequence motif UAUG, which is present in one third of all identified binding sites. The motif occurs mostly in binding sites mapping to the Open Reading Frame (ORF) and start codons and is bound by the third RRM domain (RRM3). Mutations within RRM3 however do not interfere with hyphal growth, whereas mutating the first RRM domain leads to a loss of function of Rrm4. This indicates that the RRM domains differentially contribute to RNA binding and may selectively affect distinct

mRNA target sets. However, the principles of RNA recognition by multi-domain RBPs are not well studied. Therefore, we started - to dissect the binding behavior of the different RRM domains of Rrm4 by applying *in vivo* UV-crosslinking to alleles of Rrm4 carrying mutations in their RRM domains. This should enable us to further characterize their roles during endosomal mRNA transport.

---

## 109-MCBP

Classification of small RNAs with unknown function in *B. subtilis*

\*M. Schedlowski<sup>1</sup>, P. Hildebrandt<sup>1</sup>, S. Michalik<sup>1</sup>, U. Völker<sup>1</sup>, A. Reder<sup>1</sup>

<sup>1</sup>University Medicine Greifswald, Functional Genomics, Greifswald, Germany

To fully understand global transcriptome data the functionality of each transcription unit has to be characterized. Especially small transcripts, potentially encoding very small proteins or regulatory RNAs are difficult to characterize. Nevertheless, these small effector molecules might be a missing link in understanding fundamental aspects of major life processes and therefore have to be investigated systematically. Omics approaches do provide very good basic data but do not displace detailed and focused functional *in vivo* analyzes. We started the current study with the aim to establish an efficient way to differentiate transcription units with unknown functions into non-coding and coding RNAs. Therefore, we created the plasmid based screening system pMAX\_TREP for the overexpression of transcribed genomic loci in *B. subtilis*. The pMAX\_TREP system incorporates multiple elements allowing the combination of diverse features e.g. an efficient and fast cloning of desired features via the SLIC In-Fusion system, unidirectional replication and transcription, tight target promoter repression, easy screening and real time quantifications via a CFP reporter gene expression and very quick purification by a C-terminal TWIN-STREP-tag (IBA) for subsequent identification and proof by mass spectrometry in one workflow. Furthermore, the tight transcriptional control as well as the translational fusion of the target feature to the reporter gene-Strep construct allows a broad variety of functional follow up experiments like protein-protein-interaction analyses via SPINE or global transcriptome and proteome experiments. The pMAX\_TREP system was successfully tested on 5 small RNAs of *B. subtilis* with unknown function. This first pilot experiments already provided phenotypic data as well as explicit division into coding (small proteins) and non-coding RNAs.

---

## 110-MCBP

A handy tool for *in vivo* characterization of promoter elements in *Bacillus subtilis*.

\*M. Harms<sup>1</sup>, J. P. Hildebrandt<sup>1</sup>, S. Michalik<sup>1</sup>, U. Völker<sup>1</sup>, A. Reder<sup>1</sup>

<sup>1</sup>University Medicine Greifswald, Institute for Genetics and Functional Genomics, Greifswald, Germany

The initiation of messenger RNA synthesis is mediated by various *cis*-acting DNA elements. The core promoter is essential for assembly and translocation of the RNA polymerase holoenzyme. However, the magnitude of gene transcription is not only depending on the core promoter. Additional promoter elements, which can be located up- and downstream of the core promoter, are able to enhance or repress the transcription efficiency. Deciphering the contribution of these different promoter elements to overall promoter strength is tedious. Therefore, we constructed a plasmid based screening system pHIS (High Screen) to easily address aspects like (i) effect of spacer length and

sequence, (ii) conserved regulatory sequences which are located up- or downstream of the core and (iii) fast efficient cloning of desired promoter variants via SLIC method. The most important features are (iv) an easy read out of promoter activity via fluorescent reporter gene expression and (v) *in vivo* analysis of all desired promoter constructs. Furthermore, the plasmid pHIS offers the opportunity to compare different biological or technical replicates through a constant internal normalization. To proof the functionality of the pHIS plasmid we characterized the promoter of the *ydbD* gene which is part of the general stress response of *B. subtilis*. The regulation of the *ydbD* gene is complex involving the alternative sigma factor SigB as well as the transcription factor MgsR. By using the pHIS plasmid a conserved upstream sequence was identified, which has a significant effect on promoter activity of *ydbD* and finally on the global transcription patterns of the entire MgsR regulon.

---

## 111-MCBP

The *Legionella pneumophila* GDSL hydrolase PlaA, a highly active lysophospholipase, is processed and activated by the zinc metalloproteinase ProA

\*M. Hiller<sup>1</sup>, C. Lang<sup>1</sup>, J. Döllinger<sup>2</sup>, M. Diwo<sup>3</sup>, W. Blankenfeldt<sup>3</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>Robert Koch Institute, Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany

<sup>2</sup>Robert Koch-Institut, Division of Proteomics and Spectroscopy, Berlin, Germany

<sup>3</sup>Helmholtz Center for Infection Research, Structure and Function of Proteins, Braunschweig, Germany

## Introduction

The facultative intracellular pathogen *Legionella pneumophila* is the causative agent of Legionnaire's disease, a potentially fatal pneumonia. *L. pneumophila* is ubiquitous in aqueous habitats and amoebae are a natural host. However, the pathogen can also colonize lung macrophages and epithelial cells. *L. pneumophila* blocks phagosome maturation and thus evades degradation in the lysosome. Instead, the *Legionella* containing vacuole (LCV) is established which allows replication of *Legionella*. During infection *L. pneumophila* secretes proteins, among others phospholipases, into the lumen of the LCV and the host cell cytoplasm via its type II and type IVB secretion systems. At least 15 phospholipases A, which divide into the patatin-like proteins, the PlaB-like proteins and the GDSL hydrolases, are encoded in the genome. The GDSL hydrolase PlaA was previously described as exit factor of *L. pneumophila*.

## Objectives

We here focus on the characterization of the phospholipase PlaA which belongs to the family of GDSL hydrolases. In the presented project we investigate the mode of action, activation mechanism and 3D structure of PlaA.

## Materials and methods

The mode of secretion was assessed by Western blotting. For detection of enzymatic activity, recombinant PlaA was purified and subjected to lipid hydrolysis assay and thin layer chromatography. Additionally the effect of the zinc metalloproteinase ProA on PlaA integrity and activity was determined. Moreover, the 3D structure of PlaA was determined via crystallization.

## Results

We showed that PlaA is secreted via the type II secretion system and exhibits strong lysophospholipase A activity. Moreover, PlaA was processed by the *L. pneumophila* zinc metalloproteinase within a disulfide loop after secretion which increases its lysophospholipase A activity. Additionally, we present the 3D structure of PlaA which revealed that the uncleaved disulfide loop stabilizes a lid covering the catalytic triad.

## Conclusion

As PlaA is secreted via the type II secretion system and thus present within the LCV lumen we propose that its lysophospholipase activity is directed towards the LCV membrane and thus promotes the exit of *L. pneumophila*.

## 112-MCBP

**In vivo** characterization of the mechanisms of antibiotics on the bacterial cell envelope

\*A. B. Schäfer<sup>1</sup>, M. Wenzel<sup>1</sup>

<sup>1</sup>Chalmers University of Technology, Department of Biology and Biological Engineering, Division of Chemical Biology, Gothenburg, Sweden

Our healthcare system is facing a major challenge due to rising multidrug resistance, causing previously easily curable infections to evolve into complicated diseases. At the same time, the discovery of new antibiotics is lacking far behind.

One promising way to tackle multi-resistant strains is to target their cell envelope. Not only constitutes the cell envelope the first line of defense against antibiotic compounds, it is also essential for the survival of the cell. Furthermore, it harbors a multitude of crucial cellular processes, which makes it sensitive to drugs. Due to the complexity of the outer membrane, cell wall, and cytoplasmic membrane resistance against compounds targeting the cell envelope develops much slower compared to compounds with single protein targets. However, to exploit this drug target more efficiently, we need to better understand its biology and how antibiotics disturb its function.

In recent years, *in vivo* techniques became more advanced, so that it is now much more feasible to investigate the effects of antimicrobial compounds on the cell wall and membrane in living bacteria. In this project, we systematically study the effects of membrane and cell wall-active antibiotics on bacteria using fluorescence microscopy and cell biological tools.

To our surprise, we found that classical antibiotics, which are assumed to have one specific intracellular target, in fact also affect the cell envelope of bacteria, especially membrane domain organization and localization of membrane-bound proteins.

These findings demonstrate, that even widespread clinically used antibiotics are not fully understood. In order to develop the next generation of effective antibacterial drugs, it is pivotal to thoroughly understand how exactly antibiotics kill bacteria.

## 113-MCBP

Posttranscriptional regulation of SAM in the alpha-proteobacterium *Sinorhizobium meliloti*

\*R. Scheuer<sup>1</sup>, H. Melior<sup>1</sup>, E. Evgenieva-Hackenberg<sup>1</sup>

<sup>1</sup>Institute of Microbiology and Molecular Biology, Gießen, Germany

Methionine is used together with ATP to synthesize S-adenosylmethionine (SAM), the main methyl donor in the cell. We have shown that in the nitrogen fixing plant symbiont *Sinorhizobium meliloti*, RNases E and J are needed for homeostasis of SAM [1]. In the *S. meliloti* 2011 mutants with mini-Tn5 insertions in the corresponding genes *rne* and *rnj* the SAM level was increased and several sRNAs were affected in gel migration, probably due to hypermethylation [1]. In bacteria the SAM biosynthesis is posttranscriptionally regulated by SAM-binding riboswitches. In several alpha-proteobacteria SAM-II riboswitch was predicted in the 5'-UTRs of particular methionine genes [2]. In *S. meliloti* SAM-II riboswitches were predicted in the 5'-UTRs of the genes *metA* and *metZ*. To address the mechanisms of posttranscriptional SAM regulation in *S. meliloti*, we analyzed *metA* and *metZ* by qRT-PCR and the corresponding liberated SAM riboswitches (sRNA Rsw) by Northern blot hybridization. We expect under high methionine conditions increased levels of sRNA Rsw and lower mRNA levels when compared to low methionine conditions. In line with this, in the wild type strain 2011 we observed in rich TY medium when compared to minimal GMX medium increased levels of the two liberated riboswitches, while the mRNA levels were decreased. In agreement with this, plasmid reporter fusions of *metA* and *metZ* (promoter region and first 6 nucleotides of ORF are fused to eGFP) showed increased eGFP expression in minimal medium. Interestingly, the PmetA-*metA::egfp* fusion, which increases the promoter copy number and the riboswitch level in the cell, led to an increase on the *metZ* sRNA Rsw level. In contrast, the PmetZ-*metZ::egfp* fusion decreased the *metA* sRNA Rsw level. Currently we are investigating the cross-talk between the *metA* and *metZ* regulation and its relation to RNases E and J in *S. meliloti* 2011.

1 Baumgardt, K., et al. (2017). Microbiology 163, 570–583.

2 Corbino, K. A., et al. (2005). Genome Biol. 6:R70

## 114-MCBP

ReoM and ReoY connect cell wall integrity surveillance with peptidoglycan biosynthesis via an essential protein phosphorylation event

\*S. Wamp<sup>1</sup>, Z. J. Rutter<sup>2</sup>, C. E. Jennings<sup>3</sup>, J. Rismondo<sup>4</sup>, R. J. Lewis<sup>2</sup>, S. Halbedel<sup>1</sup>

<sup>1</sup>Robert Koch Institute, FG11 Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany

<sup>2</sup>Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne, United Kingdom

<sup>3</sup>Newcastle Drug Discovery, Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom

<sup>4</sup>Section of Microbiology and MRC Centre for Molecular Bacteriology and Infection, Imperial College London, London, United Kingdom

In *Listeria monocytogenes* the late cell division protein GpsB is required for cytokinesis and peptidoglycan biosynthesis. These effects are explained by an interaction of GpsB with the major penicillin binding protein PBP A1, which is responsible for polymerization and cross-linking of peptidoglycan strands (1, 2). We noticed that a *L. monocytogenes*  $\Delta$ *gpsB* mutant rapidly acquires mutations suppressing the  $\Delta$ *gpsB* phenotype. A first set of these suppressor mutations has been described to increase the influx of precursor molecules into cell wall biosynthesis by proteolytic stabilization of MurA, the first enzyme of this pathway, through mutations in the protease subunit ClpC (3).

We here show that inactivation of two yet uncharacterized suppressor genes, *reoM* and *reoY*, also leads to accumulation of MurA, indicating that these genes represent novel factors contributing to ClpCP-dependent MurA



degradation. These results were supported by the elucidation of the crystal structure of one of these *gpsB* suppressor genes *reoM* (regulator of *MurA* degradation), identifying it as a homolog of IreB from *Enterococcus faecalis*, a small phosphoprotein substrate of the serine/threonine protein kinase IreK (4). *In vitro* and *in vivo* studies showed that ReoM is phosphorylated by PrkA, the corresponding homolog of the kinase in *L. monocytogenes*, and that phospho-ablative mutations in *reoM* are lethal. Furthermore, it has been observed that inactivation of the kinase PrkA or the cognate phosphatase PrkP has opposing effects on *MurA* levels in a ClpCP-dependent manner, supporting the idea that proteolytic stability of *MurA* is controlled by the PrkA/PrkP protein pair. Our data are consistent with a model in which the PrkA substrate identified here provides a novel link between peptidoglycan biosynthesis and cell wall integrity sensing. Moreover, they provide an explanation for many observations on the regulation of peptidoglycan biosynthesis by PASTA-domain containing serine/threonine protein kinases previously made in other firmicutes.

1 - Rismondo *et al.*, 2016. Mol. Microbiol.

2 - Cleverley *et al.*, 2019. Nat. Commun.

3 - Rismondo *et al.*, 2017. J. Bacteriol.

4 - Hall *et al.* 2017. J Mol Biol.

## 115-MCBP

### The benzothiazinone BTZ043 blocks apical growth and affects septum formation in *Corynebacterium glutamicum*

\*F. Meyer<sup>1,2</sup>, K. Schubert<sup>2</sup>, M. Bramkamp<sup>2</sup>

<sup>1</sup>Christian-Albrechts-Universität zu Kiel, Institut für Allgemeine Mikrobiologie, Kiel, Germany

<sup>2</sup>Ludwig-Maximilians-University Munich, Fakultät Biologie, Planegg-Martinsried, Germany

*Corynebacteria* and *Mycobacteria* share a complex, multilayered envelope architecture where a peptidoglycan layer with covalently bound arabinogalactan provides a scaffold for an out-facing bilayer composed of mycolic acids and trehalose mycolates. This results in a hydrophobic barrier against water-soluble chemicals, including some antibiotics. However, synthesis and maintenance of the cell envelope provides a potential Achilles' heel to combat these bacteria. Many first line antibiotics for tuberculosis treatment such as isoniazid and ethambutol act in the cell envelope synthesis pathway. Recent years have seen a dramatic increase in antibiotic resistance, also against these first line drugs. New compounds with different working mechanisms are therefore of special interest. One novel anti-tuberculosis drug candidate is benzothiazinone 043 (BTZ), a compound that is currently entering phase I for clinical trials. It was shown that BTZ blocks the DprE1 enzyme involved in the arabinose synthesis. However, the effect of BTZ on the single cell level and the subsequent inhibition of cell growth were not understood in detail. Here we used *Corynebacterium glutamicum* to address this question. Our results suggest that BTZ only affects one of the two distinct cell-wall synthesis machineries present in *C. glutamicum*, namely the elongasome. Whereas the apical elongation is completely stopped, the activity of the divisome is less affected. Using comprehensive imaging of fluorescently labeled nascent peptidoglycan synthesis we find that BTZ treated cells grow from active septa in a way similar to coccoidal cells. Thus our data reveal how flexible *C. glutamicum* cells adapt to the presence of cell envelope acting antibiotics.

## 116-MCBP

An archaeal / bacterial consortium from a Costa Rica petrol well: analysis by electron tomography

L. Dengler<sup>1</sup>, J. Flechsler<sup>2</sup>, A. Klingl<sup>2</sup>, H. Huber<sup>1</sup>, D. Grohmann<sup>1</sup>, \*R. Rachel<sup>3</sup>

<sup>1</sup>University of Regensburg, Dept of Microbiology and Archaea Centre, Regensburg, Germany

<sup>2</sup>LMU Munich, Plant Development, Martinsried, Germany

<sup>3</sup>University of Regensburg, Centre for Electron Microscopy, Regensburg, Germany

Microbial biofilms are widespread in nature and are often composed of several species. Biofilm formation has great impact in biotechnological applications and in medical care. Our current research aims to get a deep understanding of the biofilm composition, formation, and cell-cell interaction. In this study, we analyse a biofilm originally isolated from an ancient petrol well in Cahuita, Costa Rica. It consists of cells of the archaeal genus *Methanobacterium* and a sulfate-reducing bacterium (SRB) of the genus *Desulfomicrobium*. Both are novel species. The co-culture is stable under laboratory conditions. Our analyses revealed that the biofilm-matrix surrounding the cells is mainly produced by SRB and acts as an assembly centre for metal sulfides. Currently, we analyse the cell structure and cell-cell interaction structures by electron tomography.

Methods: Cells are analysed by electron tomographic methods (Rachel *et al.*, 2010) following cryo-sample preparation: high-pressure freezing, freeze-substitution, and resin embedding. We obtain ultrathin (70 nm) and semithin (400 nm) sections, which are analysed by transmission electron microscopy / tomography. For even thicker sections (900 nm), we employ parallel beam STEM tomography, enabling us to eliminate the focus gradient at high tilt, by increasing the depth of focus.

Results: The tomography datasets help us to visualize the cells with minimal distortion, using the improved sample preparation protocol. The natural shape of the cells is trapped in an undisturbed way, before their structure is analysed at an almost isotropic resolution of at least 5 nm. The methanogens are almost straight, rod-shaped cells, while the SRB show a cell wall with an outer membrane, enclosing an unusually wide periplasm.

Conclusion: Our tomographic analyses, together with fluorescence microscopy, physiological and biochemical analyses of this consortium exhibit a complex cell architecture and help to visualize the extracellular polymeric substances (EPS). We plan to complement our studies using electron cryo-tomography, in the near future.

## References:

Rachel *et al.*, 2010: Meth Cell Biol 96: 47-69

## 117-MCBP

Mdh (metal-dependent hydrolase): The unknown of the methionine biosynthesis operon in *Staphylococcus aureus*

\*F. D. R. Wencker<sup>1</sup>, Y. A. Ramirez<sup>2</sup>, W. Koelmel<sup>2</sup>, D. Steigerwald<sup>1</sup>, E. Rogalski<sup>1</sup>, M. Peindl<sup>1</sup>, C. Kisker<sup>2</sup>, W. Ziebuhr<sup>1</sup>

<sup>1</sup>Julius-Maximilians Universität Würzburg, Institut für Molekulare Infektionsbiologie, Würzburg, Germany

<sup>2</sup>Julius-Maximilians Universität Würzburg, Rudolf Virchow Center for Experimental Biomedicine, Institute for Structural Biology, Würzburg, Germany

The opportunistic human pathogen *S. aureus* is capable of synthesising methionine *de novo* when the amino acid becomes scarce. Genes required for methionine biosynthesis are encoded by the *met* operon whose expression exclusively takes place under methionine-deprived conditions. Next to the *met* genes (*metI*, *C*, *F* and *E*), the operon is predicted to comprise an additional gene encoding for a metal-dependent hydrolase (*mdh*) of unknown function.

Here we demonstrate co-transcription and –expression of *mdh* with the *met* operon. We succeeded in overexpressing, crystallising and solving the structure of Mdh at high resolution. Crystal structure analyses identified zinc as a cofactor and a dimeric state as preferred conformation of the protein. Amino acid residues suggested to be crucial for catalytic activity and substrate recognition were targeted, and *mdh* variants were chromosomally integrated into *S. aureus* for *in vivo* viability analysis. Interestingly, while deletion of the entire *mdh* gene had no effect, catalytic site variants were growth impaired under methionine deprived conditions. Initial protein-protein interaction studies indicated co-localization of Mdh with (some of) the methionine biosynthesis enzymes of the operon. Furthermore, the association of *mdh* with the *met* operon was found to be highly conserved among staphylococci, strongly suggesting a link with methionine metabolism. Although the substrate of Mdh is still unknown, BLAST analyses identified some similarities of Mdh with kynurenine formamidases, cleaving N-formyl-kynurenine into kynurenine and formic acid in *Pseudomonas*. Formic acid is required for formylation of methionine residues charged onto initiator-tRNAs, whose availability is decisive for bacterial translation initiation. We hypothesise that co-expression of Mdh with methionine biosynthesis enzymes might allow coupling of methionine synthesis and methionine formylation for accelerated translation initiation under methionine deprived conditions. Mdh could provide formic acid (for example) by cleaving formylated substrates such as f-methionine or f-glutamate. Experiments are currently underway to substantiate this hypothesis.

#### 118-EPAP

The composition and function of the electron transport chain catalyzing ***Wolinella succinogenes*** nitrous oxide respiration

\*C. Werner<sup>1</sup>, S. Hein<sup>1</sup>, S. Deusch<sup>2</sup>, J. Seifert<sup>2</sup>, J. Simon<sup>1</sup>

<sup>1</sup>Technische Universität Darmstadt, Department of Biology, Microbial Energy Conversion and Biotechnology, Darmstadt, Germany

<sup>2</sup>University of Hohenheim, Institute of Animal Science, Stuttgart, Germany

Over the last decades, climate change is moving more and more into public consciousness. Besides the commonly mentioned carbon dioxide and methane, nitrous oxide (N<sub>2</sub>O) is an important greenhouse gas with substantial global warming potential. Most of the anthropogenic N<sub>2</sub>O emissions derive from agricultural soils, mainly due to the application of synthetic nitrogen-containing fertilizers. The increasing concern about N<sub>2</sub>O emissions directed interest on bacterial species harbouring the copper-containing N<sub>2</sub>O reductase (NosZ), which is the only known enzyme that is able to convert N<sub>2</sub>O to N<sub>2</sub>. During anaerobic N<sub>2</sub>O respiration, NosZ acts as the terminal reductase of an electron transport chain that conserves energy through proton motive force generation. Based on phylogenetic analyses, it has been recognized that NosZ enzymes can be categorized into clade I or clade II and that this classification also applies to the genetic context of the corresponding *nos* gene clusters.

Here, we present experimental work on the N<sub>2</sub>O-respiring clade II model bacterium *Wolinella succinogenes*. Apart from periplasmic NosZ, at least five different *nos* gene cluster-

encoded proteins as well as a membrane-bound Rieske/cytochrome *bc* complex were found to be essential for N<sub>2</sub>O respiration. Transcriptome and proteome experiments will be presented that contribute to the elucidation of the assumed membrane-bound N<sub>2</sub>O respirasome.

#### 119-EPAP

The author has not agreed to a publication.

#### 120-EPAP

##### Function and phylogeny of DsrL, a NAD(P)H oxidoreductase involved in dissimilatory sulfur metabolism

\*K. Wallerang<sup>1</sup>, M. Löffler<sup>1</sup>, C. Dahl<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Mikrobiologie und Biotechnologie, Bonn, Germany

DsrAB-type dissimilatory siroheme-sulfite reductase is a key enzyme of microbial sulfur-based energy metabolism and occurs in sulfate/sulfite reducers as well as in sulfur oxidizers. The *dsr* gene clusters of sulfur oxidizers typically encode DsrL, which is essential for sulfur oxidation in *Allochromatium vinosum*. It acts as NAD(P)H oxidoreductase and physiological partner of AvDsrAB. Recent genome analyses and metagenomic studies revealed that *dsrL* is not restricted to sulfur oxidizers as previously assumed but also occurs in *dsr* clusters of (probable) sulfate/sulfur-reducing bacteria, e.g. *Desulfurella amilsii*<sup>1,2</sup>. We elucidated differences between DsrL proteins from sulfur oxidizers and from organisms reducing inorganic sulfur compounds. Phylogenetic analysis revealed early separation of reductive and oxidative DsrL types. All DsrL proteins consist of an N-terminal ferredoxin domain and a central FAD and NAD(P)-binding part linked to a second ferredoxin domain. Oxidative-type DsrLs have longer substrate binding and linker domains, while the vast majority of reductive-type DsrLs shares a conserved double arginine-motif in their substrate binding domain. The latter has been identified as binding the NADP-phosphate in the related NfnB protein<sup>3</sup>. In accordance, DsrL from *D. amilsii* is active with NADP(H) but not with NAD(H), while *A. vinosum* DsrL uses both substrates albeit with a strong preference for NAD(H). Among the oxidative-type DsrLs, the green sulfur bacterial proteins form a separate cluster. They are completely inactive with NADP(H) as shown for the enzyme from *Chlorobaculum tepidum*. In conclusion, DsrL proteins have evolved to serve specific functions in organisms oxidizing or reducing sulfur compounds. In sulfur oxidizers, NAD<sup>+</sup> appears as suitable *in vivo* electron acceptor for DsrABL-catalysed sulfur oxidation, while NADPH is required as electron donor for sulfite reduction. This observation can be related to the much lower redox potential for NADPH/NADP<sup>+</sup> as compared to the NADH/NAD<sup>+</sup> couple under physiological conditions.

<sup>1</sup>Anantharaman et al 2018 ISMEJ 12, 1715

<sup>2</sup>Florentino et al 2019 Env Microb 21, 209

<sup>3</sup>Demmer et al 2015 JBC 290, 21985

## 121-EPAP

The author has not agreed to a publication.

---

## 122-EPAP

Time-dependent transcriptome landscape of *Parageobacillus thermoglucosidasius* DSM 6285 hydrogenogenesis under carbon monoxide and air atmosphere

\*H. Aliyu<sup>1</sup>, T. Mohr<sup>1</sup>, D. Cowan<sup>2</sup>, P. De Maayer<sup>3</sup>, A. Neumann<sup>1</sup>  
<sup>1</sup>Karlsruhe Institute of Technology, Section II: Technical Biology, Institute of Process Engineering in Life Science, Karlsruhe, Germany  
<sup>2</sup>University of Pretoria, Centre for Microbial Ecology and Genomics, Pretoria, South Africa  
<sup>3</sup>University of the Witwatersrand, School of Molecular & Cell Biology Faculty of Science, Johannesburg, Germany

*Parageobacillus thermoglucosidasius* is a facultative anaerobic thermophile frequently isolated from both mesophilic and thermophilic environments. Previous genomic studies have shown that all *P. thermoglucosidasius* strains harbour three anaerobic CO dehydrogenase (CODH) and twelve H<sub>2</sub>-evolving hydrogenase genes that are co-localised on the chromosomes. Furthermore, gas analysis revealed that most of the *P. thermoglucosidasius* strains readily oxidise CO and produce H<sub>2</sub> via water-gas shift reaction (WGS) with DSM 6285 showing the highest H<sub>2</sub> production rate among the compared strains. To determine the genetic control of the WGS in *P. thermoglucosidasius* DSM 6285, total RNA was extracted from cultures grown under 50% CO and 50% air gas atmosphere at four timepoints in duplicates and the rRNA depleted fraction was sequenced using Illumina NextSeq, v2.5, 1x75bp (stranded) chemistry. The expression pattern of the transcripts was determined using DP\_GP cluster analysis. The RNA-Seq data revealed 28 distinct gene expression trajectories across the four time points. Evaluation of the trajectories of the fifteen CODH and hydrogenase genes associated with WGS revealed that the transcripts are distributed in three clusters showing similar expression pattern of an initial rapid up-regulation with O<sub>2</sub> depletion and subsequent down-regulation with decreasing CO and increasing H<sub>2</sub> concentrations. This trend suggests a possible influence of both O<sub>2</sub> and CO on the expression of the anaerobic CODH and H<sub>2</sub>-evolving hydrogenase genes. EggNOG annotation of the similarly expressed genes in the three clusters showed an overrepresentation of the functional category metabolism consistent with the DSM 6285 growth based on OD600 readings. Overall, the data revealed a clear partitioning of the transcripts that reflects the metabolic shift associated with the transition from aerobic to anaerobic metabolism and the evolution of hydrogen via water-gas shift reaction.

## References

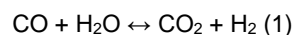
- Mohr, T. et al. *Microbial cell factories* **17**, 108 (2018)  
Mohr, T. et al. *BMC genomics* **19**, 880 (2018)
- 

## 123-EPAP

Regulation of hydrogen production in *Parageobacillus thermoglucosidasius* DSM 6285

R. Küchlin<sup>1</sup>, H. Aliyu<sup>1</sup>, D. Cowan<sup>2</sup>, P. De Maayer<sup>3</sup>, \*A. Neumann<sup>1</sup>  
<sup>1</sup>Karlsruhe Institut of Technology, Technical Biology, Karlsruhe, Germany  
<sup>2</sup>University of Pretoria, Centre for Microbial Ecology and Genomics, Pretoria, South Africa  
<sup>3</sup>University of the Witwatersrand, School of Molecular & Cell Biology, Johannesburg, South Africa

Motivated by dwindling fossil resources and progressive climate change, the search for alternative energy sources is in full swing. Hydrogen is an energy carrier with more than twice the gravimetric energy density of conventional natural gas. *Parageobacillus thermoglucosidasius* DSM 6285 is a gram-positive, hydrogen-forming, facultative anaerobic bacterium. It can grow in the presence of high concentration of carbon monoxide and catalyzes the water gas shift reaction (1) for the production of H<sub>2</sub>. Genomic analyses showed that this organism contains the genes for a [Ni-Fe] group 4a hydrogenase forming a complex with the anaerobic (Coo) CO dehydrogenase.<sup>1,2</sup>



For extended biotechnological application a profound understanding of the regulation of the water gas shift reaction is a prerequisite. Therefore the gas conversion as well as growth and activity of the carbon monoxide dehydrogenase (CO-DH) was monitored in batch experiments with and without CO in the gas phase. The transcription of three CO-DH and two hydrogenase genes were examined with RT-qPCR. Activity of the CO-DH was measured with whole cells as the reduction of methyl viologen in the presence of CO.

In batch experiments with CO in the gas phase three different phases were distinguishable: i an aerobic growth phase with no conversion of CO; ii a lag phase with complete depletion of oxygen but no growth; iii a hydrogen production phase at about constant OD and an increase in CO-DH activity. In batch experiments without CO growth in phase one was similar but there was a sharp decrease in OD in phase two. After complete depletion of oxygen in phase three, OD decline was slower but unexpectedly activity of CO-DH was measureable. RT-qPCR data for the CO-DH genes supported the observed activity profile of the CO-DH.

The expression of the CO-DH seems to be inhibited in the presence of oxygen. Under anoxic conditions the expression of the CO-DH is induced but specific activity is significantly higher with CO in the gas phase.

- <sup>1</sup>Mohr T, et al (2018) *Microb Cell Fact* doi: 10.1186/s12934-018-0954-3  
<sup>2</sup>Mohr T, et al (2018) *BMC Genomics*. doi: 10.1186/s12864-018-5302-9
- 

## 124-EPAP

Is the formate dehydrogenase H of *Escherichia coli* promiscuous?

\*D. Lubek<sup>1</sup>, C. Pinske<sup>1</sup>  
<sup>1</sup>Martin-Luther-University Halle-Wittenberg, Biology/Microbiology, Halle (Saale), Germany

The ubiquitously distributed small subunits of redox enzymes harbor iron-sulfur clusters for electron transfer and the protein fold often resembles that of the electron-transferring ferredoxins. Direct protein interactions ensure that the maximum electron-transfer distance of about 13 Å is maintained. Two of the 25 ferredoxin-like proteins in *Escherichia coli* are located within the formate hydrogenlyase (FHL) complex, which catalyzes the oxidation of formate to CO<sub>2</sub> through the formate dehydrogenase H (FDH-H) enzyme with concomitant production of H<sub>2</sub> through proton reduction via a hydrogenase (Hyd-3). It has been shown that the FDH-H protein can also interact with an alternative FHL complex<sup>1</sup>. This helps explain why the encoding *fdhF* gene is not part of the *hyc* operon encoding Hyd-3, but is co-regulated with it. A recent study identified an

interaction network of ferredoxin-like proteins and identified YsaA and HydN as novel interaction partners of the FDH-H protein by bacterial two hybrid assays and through impaired FDH-H activity in *ysaA* and *hydN* deletion strains<sup>2</sup>.

Using protein interaction studies we aim to verify the findings of the bacterial-two-hybrid interaction, shed light on the electron pathway involved and understand the physiological role of this protein interplay.

Essentially nothing is known about the functions of YsaA and HydN. Initially, we studied *ysaA* gene expression and found that in contrast to *fdhF*, it is not regulated by formate, but rather is constitutively expressed. Affinity chromatography and optimization of the expression conditions allowed the isolation of correctly folded iron-sulfur cluster-carrying HydN, YsaA and FDH-H proteins, which serve as the basis for pull-down experiments and for quantification of the interaction strength.

Taken together, our findings suggest that the highly conserved FDH-H protein might provide reducing equivalents to several electron-transfer complexes, which ultimately likely increases the metabolic flexibility of the cell. This makes both FDH-H and these ferredoxin-like proteins useful future drug targets.

<sup>1</sup>Lindenstrauß & Pinske *J Bacteriol* 201:1480; **2019**

<sup>2</sup>Pinske *Front. Microbiol.* 9:1238; **2018**

---

## 125-EPAP

### Laboratory evolution of *Thermoanaerobacter kivui* towards reduced growth temperatures

\*M. Lehmann<sup>1</sup>, E. Zschaubitz<sup>1</sup>, M. Basen<sup>1</sup>

<sup>1</sup>University of Rostock, Microbiology, Rostock, Germany

Life on earth might have originated in a hydrothermal setting with the last universal common ancestor (LUCA) being an autotrophic thermophilic anaerobe using the Wood-Ljungdahl pathway for CO<sub>2</sub> fixation [1]. Proceeding from a thermophilic LUCA growing optimally at temperatures over 45 °C, organisms that grow at moderate temperatures (mesophiles) possibly emerged out of a thermophilic lifestyle. This would require alterations regarding membrane structure or stability of nucleic acids and proteins. *Thermoanaerobacter kivui* is the most thermophilic acetogenic bacterium characterized yet, growing optimally at 66 °C and using the Wood-Ljungdahl pathway to produce acetate [2]. Its physiology possibly resembles that of LUCA, making it a perfect model organism to study the evolutionary changes necessary to grow at moderate temperatures. Here, we show first results of the adaptive laboratory evolution towards a reduced optimal growth temperature of *T. kivui* using continuous culture and serial passaging, respectively. In continuous culture, we established a steady state at 66 °C with subsequent decrease of the incubation temperature. Surprisingly, the culture's response to lower temperatures was quite robust resulting in a rather stable optical density. Especially, morphological changes of the cells were noticeable after incubation at temperatures below 50 °C. This could be observed during passaging experiments as well. Serial passaging resulted in an isolate that indeed showed adaption to reduced growth temperatures, indicated by a higher growth rate and a shorter lag phase at lower temperature than the type strain. The results from these experiments lead to the conclusion that *T. kivui* responds rapidly to suboptimal temperatures. Currently, we are

studying the genotypic and phenotypic changes in the adapted strain.

[1] Weiss MC, Sousa FL, Mrnjavac N, *et al. Nat Microbiol.* 2016 Jul 25;1(9):16116.

[2] Basen M, Müller V. *Extremophiles.* 2017 Jan;21(1):15-26.

---

## 126-EPAP

### New insights into the substrate determinants of benzylsuccinate synthase (BSS) by analysing mutated variants

\*A. Alhaj Zein<sup>1</sup>

<sup>1</sup>Philipps-Universität Marburg, Biologie, Marburg, Germany

In recent years, anaerobic toluene degradation has been shown to proceed by a common pathway in various unrelated groups of bacteria which couple toluene degradation to anaerobic respiration or fermentation in syntrophic cocultures [1] [2]. The pathway is initiated by (R) - Benzylsuccinate Synthase (BSS), which belongs to the family of glycyl radical enzymes and contains a conserved glycine residue close to the C-terminus, which is converted to a glycyl radical moiety by an activating enzyme [3]. The Enzyme is a heterohexamer of  $\alpha_2\beta_2\gamma_2$  composition and catalyzes the addition of the methyl group of toluene to the double bond of a fumarate cosubstrate to form (R) - Benzylsuccinate as first intermediate of the degradation pathway [4] [5]. BSS can be regarded as a prototype enzyme for many other fumarate-adding enzymes (FAE) initialising anaerobic degradation of other hydrocarbons, such as xylenes, cresols, methylnaphthalene, or alkanes.

In our study, we established the first recombinant production system for activated BSS and used this system to construct several mutants affected in the active site in the alpha subunit of the enzyme. We show that a single mutation is sufficient to generate a variant with expanded substrate range that converts m-xylene in addition to the previously recognized substrates toluene and the cresol isomers. In accordance to the predicted effects, the constructed variant specifically turns over m-xylene and does not convert o- or p-xylene. In our ongoing study, we are also creating and characterizing additional mutant BSS variants to understand more of the reaction mechanism of BSS and to expand or alter its substrate range for potential applications. Moreover, we are also trying to recombinantly produce and purify other fumarate-adding enzymes for further biochemical and structural characterization.

[1] Rabus and Widdel, 1995.164: 448-451.

[2] Zengler, Heider et al.,1999.172: 204-212.

[3] Funk MA, *et al. Proc Natl Acad Sci USA* 2014; 111: 10161–10166.

[4] Heider J, Spormann AM, Beller HR, Widdel F. *FEMS Microbiol Rev* 1999; 22: 459–473.

[5] Verfurth K, Pierik AJ, Leutwein C, Zorn S, Heider J. *Arch Microbiol* (2004) 181 : 155–162.

---

## 127-EPAP

The emerging role of aldehyde:ferredoxin oxidoreductase in microbially-catalyzed alcohol production

\*L. Nissen<sup>1</sup>, J. Moon<sup>2</sup>, M. Basen<sup>1,2</sup>

<sup>1</sup>University Rostock, Microbiology, Rostock, Germany

<sup>2</sup>Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt, Germany

With the increasing need for alternative liquid fuels and reduction of CO<sub>2</sub> emissions, production of alcohols from biomass and from synthesis gas (mainly H<sub>2</sub>, CO, CO<sub>2</sub>) becomes important. Promising whole-cell biocatalysts are anaerobic microorganisms that utilize a pathway involving aldehyde:ferredoxin oxidoreductase (AOR). This enzyme catalyzes the reduction of organic acids to aldehydes with reduced ferredoxin. The aldehydes are then reduced to the corresponding alcohol by an alcohol dehydrogenase (ADH). AORs are oxygen-sensitive proteins containing tungsten, which is not widespread in biological systems, and they have wide substrate spectra reducing aliphatic, branched-chained aliphatic and aromatic acids.

While first hints for the AOR-ADH pathway came from Clostridia (1), it has recently been demonstrated using genetics that production of various alcohols from their corresponding acids in an engineered strain of *Pyrococcus furiosus* is catalyzed by AOR-ADH (2). Currently, evidence increases that this pathway is widespread and of biotechnological importance. A variety of microorganisms with *aor* genes present were compared in their alcohol production with regard to yield, efficiency and productivity (3).

Among the candidates which may use an AOR-ADH pathway for alcohol production, are some *Thermoanaerobacter* species. *Thermoanaerobacter* spp. are thermophilic sugar fermenters well known for their high ethanol yields; with one known exception: the acetogen *Thermoanaerobacter kivui* (2). Since *T. kivui* is genetically available and has no *aor* in its genome, the *aor* gene from *Thermoanaerobacter* sp. strain X514 was cloned into *T. kivui* for heterologous expression, with the goal to purify and biochemically characterize the protein

With the emerging role of AOR in biological alcohol production, it is important to learn more about the biochemical properties of these tungstoenzymes, also with regard to possible applications.

## REFERENZEN

(1) White, et al. 1989. *European Journal of Biochemistry* 184: 89-96.

(2) Basen et al. 2014. *PNAS* 111: 17618–17623.

(3) Nissen & Basen. 2019. *Journal of Biotechnology* 306: 105-117.

(4) Basen & Müller. 2017. *Extremophiles* 21: 15-26.

## 128-EPAP

Insights into structure and function of a benzene-mineralizing, nitrate-reducing consortium

\*S. Eziuzor<sup>1</sup>, F. Corrêa<sup>2</sup>, S. Peng<sup>3</sup>, J. Schultz<sup>4</sup>, S. Kleinsteuber<sup>2</sup>, U. Nunes da Rocha<sup>2</sup>, L. Adrian<sup>1</sup>, C. Vogt<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Isotope Biogeochemistry, Leipzig, Germany

<sup>2</sup>Helmholtz Centre for Environmental Research - UFZ, Department of

Environmental Microbiology, Leipzig, Germany

<sup>3</sup>Chongqing University, Department of Environmental Science, Chongqing, China

<sup>4</sup>Universidade Federal do Rio de Janeiro, Departamento de Microbiologia Geral, Rio de Janeiro, Brazil

The pathway how benzene is metabolized by microbes under anoxic conditions is not fully understood. Here, we studied the degradation pathway of a benzene-mineralizing, nitrate-reducing enrichment culture using a multi-omics approach. Benzene mineralization pattern and microbial community dynamics were determined in a time-series experiment using 25% fully labelled <sup>13</sup>C-benzene as sole source of carbon and energy. Benzene mineralization was dependent on the presence of nitrate and started after a lag-phase of around 70 days; benzene was mineralized at rates of 21.9±0.04 μM d<sup>-1</sup>. DNA was extracted at four different time points during mineralization and sequenced for 16S rRNA gene amplicons and shotgun-metagenome. Likewise, proteins were extracted and analyzed using liquid chromatography coupled to an Orbitrap Fusion mass spectrometer. Amplicon sequencing of 16S rRNA gene showed that the consortium was dominated by Betaproteobacteriales, Ignavibacteriales and Anaerolineales at time points of highest mineralization. A putative anaerobic benzene carboxylase was identified among other proteins, indicating that benzene is activated via carboxylation.

## 129-EPAP

Flavodoxin hydroquinone provides electrons for the ATP-dependent reactivation of protein-bound corrinoid cofactors

L. Kißling<sup>1</sup>, Y. Greiser<sup>1</sup>, H. Dürichen<sup>1</sup>, \*S. Studenik<sup>1</sup>

<sup>1</sup>Friedrich-Schiller-Universität Jena, Jena, Germany

## Introduction & Objectives

Corrinoid-dependent enzyme systems rely on the super-reduced state of the protein-bound corrinoid cofactor to be functional, e.g. in methyl transfer reactions. Due to the low redox potential of the [Co<sup>II</sup>]/Co<sup>I</sup>-couple, autoxidation of the corrinoid cofactor occurs and leads to the formation of the inactive [Co<sup>II</sup>]-state. For the reactivation, which is an energy-demanding process, electrons have to be transferred from a physiological donor to the corrinoid cofactor by the help of a reductive activator protein. In this study we elucidated the reaction sequence by which low-potential electrons are generated *in vivo* and transferred to the protein-bound corrinoid cofactors of bacterial O-demethylases.

## Materials & Methods

All proteins used in this study were heterologously produced in *Escherichia coli* as C-terminal Strep<sup>®</sup>-tag fusions. The enzyme activities were measured photometrically under anoxic conditions. The midpoint potential of flavodoxin was determined via redox titration with titanium(III) citrate as electron donor.

## Results & Conclusion

We identified reduced flavodoxin as electron donor for the ATP-dependent reduction of protein-bound corrinoid cofactors of bacterial O-demethylases. Reduced flavodoxin was generated enzymatically using pyruvate:ferredoxin/flavodoxin oxidoreductase rather than hydrogenase. Two of the four flavodoxins identified in *Acetobacterium dehalogenans* and *Desulfotobacterium hafniense* DCB-2 were functional in supplying electrons for corrinoid reduction. They exhibited a midpoint potential of about -400 mV (E<sub>SHE</sub>, pH 7.5) for the

semiquinone/hydroquinone transition. Reduced flavodoxin could be replaced by reduced clostridial ferredoxin. It was shown that the low-potential electrons of reduced flavodoxin are first transferred to the iron-sulfur cluster of the reductive activator and finally to the protein-bound corrinoid cofactor. This study further highlights the importance of reduced flavodoxin, which allows maintaining a variety of enzymatic reaction cycles by delivering low potential electrons.

---

### 130-EPAP

#### Insights into the mechanism of ketone carboxylases

\*A. C. Böhringer<sup>1</sup>, J. Heider<sup>1</sup>, K. Schühle<sup>1</sup>

<sup>1</sup>Philipps-Universität Marburg, Marburg, Germany

*Aromatoleum aromaticum* is a metabolically versatile denitrifying  $\beta$ -proteobacterium, which is able to degrade ketones like acetone or acetophenone under oxic or anoxic conditions. The key-enzyme of acetone metabolism is the acetone carboxylase (AcxABC), which forms acetoacetate by ATP-dependent carboxylation of acetone. The enzyme consists of three subunits (85/75/20 kDa) and is arranged as hexamer (Schühle and Heider, 2012). In a first step, the  $\beta$ -subunit phosphorylates acetone and  $\text{HCO}_3^-$  and thereby creates the highly reactive intermediates phosphoenolacetone and carboxyphosphate. The intermediates have to be translocated over 30 Å to the  $\alpha$ -subunit to react at a  $\text{Fe}^{2+}$ -ion of the active site to form acetoacetate. It is proposed that the enzyme forms a tunnel between the  $\alpha$ -subunit and the  $\beta$ -subunit to transfer and protect the intermediates from hydrolysis (Mus *et al.*, 2017). The substrate spectrum of the enzyme seems to be limited by the size of the tunnel. While ATPase activity is observed with different branched and elongated ketone-substrates, carboxylation activity is only possible with butanone and acetone (Schühle and Heider, 2012). Acetoacetate is further activated to acetoacetyl-CoA, which can be split to two molecules of acetyl-CoA. A succinyl-CoA: 3-ketoacid CoA-transferase or an acetoacetate-CoA-ligase maybe is involved in the activation of acetoacetate, which are both encoded in the same gene cluster as AcxABC. We investigate AcxABC with regards to the structure and function relation and the proposed mechanisms of substrate-channeling and compare it to acetone carboxylases from different organisms.

Mus, F., Eilers, B. J., Alleman, A. B., Kabasakal, B. V., Wells, J. N., Murray, J. W., Nocek, B. P., DuBois, J. L. & Peters, J. W. (2017): Structural basis for the mechanism of ATP-dependent acetone carboxylation. *Scientific reports*, 7(1), 7234.

Schühle, K., & Heider, J. (2012): Acetone and butanone metabolism of the denitrifying bacterium "*Aromatoleum aromaticum*" demonstrates novel biochemical properties of an ATP-dependent aliphatic ketone carboxylase. *Journal of bacteriology*, 194(1), 131-141

---

### 131-EPAP

#### Experiments on the Structure-Function Relationship of Aldehyde Oxidoreductase of *Aromatoleum aromaticum*

\*D. Hege<sup>1</sup>, F. Arndt<sup>1</sup>, J. Heider<sup>1</sup>

<sup>1</sup>Philipps-Universität Marburg, Biologie, Marburg, Germany

Aldehyde Oxidoreductase (AOR) from *Aromatoleum aromaticum* EbN1 is a tungsten containing enzyme and belongs to the subfamily of bacterial AORs. It was first isolated from cultures of *Aromatoleum aromaticum* that were grown anaerobically on phenylalanine and catalyzes the oxidation of a broad spectrum of different aldehydes to the

corresponding carbonic acids [1]. While only low amounts of AOR are present under standard growth conditions, a deletion strain lacking phenylacetaldehyde dehydrogenase (SR74*pdh*) has been shown to produce high amounts of active AOR which compensates for the missing enzyme during the degradation of phenylalanine [3]. Therefore, it is assumed that the physiological function of AOR is the elimination of toxic aldehyde intermediates [2].

AOR consists of three different subunits which form a  $\alpha_2\beta_2\gamma_2$  heterohexamer. The catalytic site is located in the largest subunit AorB and contains a tungsten bis-molybdopterin cofactor. Electrons are proposed to be transferred via a  $\text{Fe}_4\text{S}_4$ -cluster in AorB and four further  $\text{Fe}_4\text{S}_4$ -clusters in AorA to a FAD molecule in the subunit AorC. *In vitro* AOR uses either  $\text{NAD}^+$  or benzyl viologen as electron acceptor which suggests that  $\text{NAD}^+$  or ferredoxin are the natural electron acceptor [2].

We recently developed a heterologous expression system that allows recombinant expression of highly active affinity-tagged AOR. We will perform further studies including site-directed mutagenesis to validate the proposed electron transport mechanisms as well as structure biological approaches.

[1] Debнар-Daumler et al. (2014) *J. Bacteriol.* 196:2 438-492

[2] Arndt et al. (2019) *Front. Microbiol.* 10:71

[3] Schmitt et al. (2017) *J. Bacteriol.* 199:e00383-17

---

### 132-EPAP

#### Lactate metabolism in the acetogenic bacterium *Moorella thermoacetica*

\*F. Rosenbaum<sup>1</sup>, M. Schölmerich<sup>1</sup>

<sup>1</sup>University of Hamburg, Microbiology & Biotechnology, Hamburg, Germany

**Introduction:** Lactate is a common substrate for many strictly anaerobic microorganisms, but the biochemistry and bioenergetics of lactate oxidation is obscure. This is because the redox potential of the lactate/pyruvate pair is  $E_0' = -190$  mV, excluding a direct reduction of  $\text{NAD}^+$  ( $E_0' = -320$  mV). Some organisms circumvent this by using an electron-confining lactate dehydrogenase/electron transfer flavoprotein (LDH/Etf) complex that couples lactate oxidation to the simultaneous exergonic electron flow from reduced ferredoxin to  $\text{NAD}^+$  [1]. *Moorella thermoacetica* is an acetogenic bacterium that is able to grow on lactate, but it does not possess genes encoding an LDH/Etf complex. Hence, lactate metabolism must occur via another mechanism in this organism.

**Objectives:** The objective of this work was to assess growth of *M. thermoacetica* DSM521 on lactate and unravel how lactate is metabolised in this acetogen.

**Materials & methods:** Growth experiments and experiments with resting cells were performed to elucidate lactate metabolism.

**Results:** *M. thermoacetica* was able to grow on lactate after an adaptation. L- but not D-lactate sustained growth. Growth on lactate was stimulated by  $\text{CO}_2$ . Subsequent experiments with resting cells revealed that lactate could only be metabolised by cells prepared from cultures pre-grown on lactate, but not glucose, indicating that lactate metabolism is regulated. The cells metabolised lactate to acetate at a ratio of 2:3 in bicarbonate-buffered medium and lactate was not converted in the absence of  $\text{CO}_2$ . Furthermore, experiments

at resting cells with uncoupling agents indicate that lactate oxidation is coupled to the chemiosmotic gradient.

**Conclusion:** *M. thermoacetica* can grow on and metabolise L-lactate. Lactate oxidation leads to acetate formation and is coupled to the chemiosmotic gradient.

[1] M.C. Weghoff, J. Bertsch and V. Müller (2014) *Environ Microbiol* **17**:670-677.

---

### 133-EPAP

Calorespirometry as Real Time Indicator in Multi-Phase Anaerobic Biofuel Productions Processes, Developed at the Example of Acetone-Buthanol-Ethanol (ABE) fermentation

\*S. Paufler<sup>1</sup>, H. Sträuber<sup>1</sup>, T. Maskow<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany

**Question:** Anaerobic industrial biosynthesis are of growing importance because only little Gibbs energy of the raw material is wasted as heat due to a lower degree of oxidation of the products. Metabolic heat in combination with off-gas measurements known as calorespirometry reflects changes in stoichiometry and kinetics of bioconversions immediately, making it an ideal the parameter for controlling bioprocesses in order to increase productivity, product quality, and process stability. But how well are these tiny amounts of heat measurable, what information can be obtained from them and can this also be transferred to anaerobic biotechnology?

**Methods:** To answer these questions, the heat production rate, the gas production and the rates of the educts and products for acetone-butanol-ethanol (ABE) fermentation of *Clostridium acetobutylicum* ATCC 824 were monitored in a bioreactor. Thermokinetic models were developed to calculate the metabolic fluxes in real time from the monitored on-line data (heat and gas evolution).

**Results/Conclusions:** Changes between different metabolic phases can easily be retraced from both, the heat signal and the gas evolution. However, small errors in chemical analysis of intermediates and products lead to huge differences in the predicted heat generation. The calorimetric measurement provides more accurate process information. In combination with the off-gas analysis (calorespirometry) the calculation of metabolic fluxes was successful at least in the first production phase (i.e. acidogenesis). Initial numerical estimates indicate that calorespirometry can also be applied on an industrial scale, because with each upscaling the ratio of heat-producing volume to heat-exchanging surface increases and so does the accuracy of measurement.

---

### 134-EPAP

Cysteine metabolism in the acetogenic bacterium *Moorella thermoacetica*

\*L. Göbbels<sup>1</sup>, M. Schölmerich<sup>1</sup>

<sup>1</sup>University of Hamburg, Microbiology & Biotechnology, Hamburg, Germany

**Introduction:** It was recently reported that the acetogenic bacterium *Moorella thermoacetica* can use light energy to fuel CO<sub>2</sub> fixation when it is coated in cadmium sulfide nanoparticles (CdS) [1]. This bioinorganic hybrid system relies on cysteine as a sacrificial reductant, producing cystine in a chemical process. At the same time, cysteine is an amino acid that is central to numerous cellular processes in microorganisms. A biological conversion of cysteine in this

system is thus feasible and so far unelucidated in *M. thermoacetica* and other acetogens.

**Objectives:** The objective of this work was to shed light on aspects of the bioinorganic hybrid system with a particular focus on the fate of cysteine.

**Materials & methods:** Growth and resting cell experiments were performed to assess the bioinorganic hybrid system and cysteine metabolism in *M. thermoacetica* DSM521. Furthermore, key enzyme activities were measured in crude extracts of normal cells and cells covered in CdS.

**Results:** Experiments with resting cells revealed that both normal cells as well as cells covered in CdS produced acetate from cysteine. This process was independent of light. Acetate production increased with increasing cysteine concentrations and CO<sub>2</sub> was not required. Acetate was also produced from cystine, but this was tied to the presence of molecular H<sub>2</sub>. Growth experiments revealed that neither cadmium nor light energy affected growth of *M. thermoacetica* and cysteine alone did not support growth.

**Conclusion:** *M. thermoacetica* can use cysteine or H<sub>2</sub> + cystine as energy and carbon source to make acetate.

[1] K. K. Sakimoto, A. B. Wong and P. Yang (2016) *Science* **351**:74-77.

---

### 135-EPAP

Methylcitrate cycle enzymes in sulfur-reducing deltaproteobacteria *Desulfurella* spp.

\*E. Pettinato<sup>1</sup>, L. Steffens<sup>1</sup>, A. Mall<sup>1</sup>, I. Berg<sup>1</sup>

<sup>1</sup>Westfälische Wilhelms-Universität Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany

*Desulfurella acetivorans* is an obligate anaerobe capable to autotrophic and heterotrophic growth using the reversed oxidative TCA (roTCA) cycle with citrate synthase (CS) for both acetyl-CoA oxidation or acetyl-CoA formation from inorganic carbon [1]. Its genome contains three citrate synthase genes [1, 2]. Here we show that one of these genes encodes methylcitrate synthase which is active with both acetyl-CoA and propionyl-CoA (with  $V_{max}$  of 25 and 7.4  $\mu\text{mol min}^{-1} \text{mg}^{-1}$  and  $K_M$  of 14 and 23  $\mu\text{M}$ , respectively). Methylcitrate synthase is a specific enzyme of the methylcitrate cycle of propionate oxidation, and the corresponding *D. acetivorans* gene is indeed co-localized with three putative genes for specific enzymes of the cycle: methylcitrate dehydratase, methylaconitate hydratase and methylisocitrate lyase. Although *D. acetivorans* did not grow with propionate as the sole carbon source, suggesting the involvement of these enzymes in housekeeping metabolism of odd-chain fatty acids, another species of this genus, *D. propionica* [3], is capable to grow on propionate and thus possesses a fully operational methylcitrate cycle.

[1] A. Mall *et al.*, *Science* **359**, 563-567 (2018).

[2] L. Steffens *et al.*, VAAM (2020).

[3] M. L. Miroshnichenko *et al.*, *IJSB* **48**, 475-479 (1998).

---

### 136-EPAP

Biodiversity of denitrifying fungi revisited: New nitrous oxide producing isolates

\*T. Spanner<sup>1</sup>, U. Behrendt<sup>2</sup>, T. Timsy<sup>2</sup>, A. Ulrich<sup>2</sup>, S. Kolb<sup>2</sup>, M. A. Horn<sup>1</sup>

<sup>1</sup>Leibniz University Hannover, Institute of Microbiology, Hannover, Germany

<sup>2</sup>Leibniz Centre for Agricultural Landscape Research (ZALF), Muencheberg, Germany

N<sub>2</sub>O is a potent greenhouse gas with a global warming potential of app. 300x that of CO<sub>2</sub> and is mostly produced in agriculture. Grasslands under agricultural use are hotspots for N<sub>2</sub>O production due to the input of reactive nitrogen compounds like nitrate and ammonium by fertilizer application. Nitrate and ammonia are keystones in the nitrogen cycle. N<sub>2</sub>O is a byproduct and intermediate from dissimilatory nitrate reduction to ammonium (DNRA) and denitrification, respectively. Denitrification *sensu stricto* is the stepwise reduction of nitrite via nitric oxide to N<sub>2</sub>O. Optional reaction modules include nitrate reduction to nitrite and N<sub>2</sub>O reduction to molecular nitrogen. Many bacteria from different phyla are known to denitrify whereas for fungi only 95 genera are known to denitrify. Fungal denitrifiers known to date lack N<sub>2</sub>O reductases. Despite their potential to produce N<sub>2</sub>O, their role for N<sub>2</sub>O emissions is largely debated. To broaden the knowledge about denitrifying fungi, we isolated more than 300 fungi from the top soil of eight different agriculturally used grasslands under conditions favoring denitrification (i.e., anoxia, organic electron donors, and nitrate). We obtained isolates from 38 different genera as indicated by ITS analysis and tested them for their ability to denitrify. The genera *Fusarium* and *Chlonostachys* contained the most potent N<sub>2</sub>O producers. N<sub>2</sub>O producing species in 20 genera like *Westerdykella* and *Apiotrichum* that were hitherto unknown to denitrify were identified. N<sub>2</sub>O production in fungal isolates was often associated with the production of significant amounts of nitric oxide, which is a toxic intermediate of the denitrification pathway and usually undetected because of a high turnover rate to N<sub>2</sub>O. Most nitric oxide was produced by species belonging to the genera *Penicillium*, *Chlonostachys* and *Trichoderma*. Thus, we expanded the known biodiversity of denitrifying fungi from grasslands and identified hitherto underappreciated physiological traits relevant to climate change.

### 137-EPAP

Bacterial microcompartments in *Bilophila wadsworthia* for taurine metabolism and organosulfonate respiration

\*A. Burrichter<sup>1</sup>, S. Dörr<sup>1</sup>, S. Haiß<sup>1</sup>, D. Schleheck<sup>1</sup>

<sup>1</sup>Universität Konstanz, FB Biologie, Konstanz, Germany

The strictly anaerobic, sulfite-reducing bacterium *Bilophila wadsworthia* is a normal member of the human-gut microbial community but has also been associated with the development of diseases such as appendicitis and colitis. *B. wadsworthia* is specialized on the utilization of organosulfonates such as taurine (2-aminoethanesulfonate) as sulfite donors for sulfite respiration (thus, 'organosulfonate respiration'), producing harmful hydrogen sulfide (H<sub>2</sub>S). The taurine desulfonation pathway of *B. wadsworthia* was recently shown to involve a glycyl radical enzyme (GRE), isethionate sulfite-lyase (IslAB), which cleaves isethionate (2-hydroxyethanesulfonate) into toxic acetaldehyde and sulfite. Here we show that this reaction takes place isolated within bacterial microcompartments (BMCs). First, we confirmed an inducible production of BMCs during growth with taurine by proteomic, transcriptomic and electron microscopical analyses. Then, we isolated the BMCs from taurine-grown cells by density-gradient ultracentrifugation and analyzed their composition by proteomics as well as by enzymes assays, which suggested that the GRE reaction and the further conversion of the acetaldehyde take place within the BMCs. Hence, we characterized a novel subclass of BMCs and also provide more details on biomedically

relevant microbial organosulfonate respiration and H<sub>2</sub>S formation.

### 138-EPAP

HybG-HypD scaffold complex of the [NiFe]-Hydrogenases Maturation Machinery has an ATPase activity

\*A. Haase<sup>1</sup>, K. Nutschan<sup>1</sup>, R. Golbik<sup>2</sup>, G. Sawers<sup>1</sup>

<sup>1</sup>Martin-Luther-University Halle-Wittenberg, Institute of Biology/Microbiology, Halle (Saale), Germany

<sup>2</sup>Martin-Luther-University Halle-Wittenberg, Institute of Biochemistry and Biotechnology, Halle (Saale), Germany

[NiFe]-hydrogenases are iron sulphur-cluster containing enzymes and have an unusual bimetallic NiFe(CN)<sub>2</sub>CO cofactor in the active site of the large subunit. Six conserved Hyp proteins (HypA-HypF; HybG as a HypC isoform in *E. coli*) are required for assembly of the bimetallic cofactor. While the CN<sup>-</sup> ligands are generated from carbamoylphosphate by the HypEF proteins, the origin of the CO ligand remains unclear. The (FeS)-cluster-containing HypD protein is the only one that is redox-active and it likely plays a key role in the biosynthesis of the CO ligand. The CO ligand might originate from endogenously produced CO<sub>2</sub> already bound to an Fe ion on a HypD-HybG sub-complex [1]. The reduction of CO<sub>2</sub> to CO, however, requires a redox potential of approximately -535 mV (in solution), but the redox potential of the FeS-Cluster of HypD (E<sup>0</sup> = -260 mV) [2] is too positive to be able to reduce CO<sub>2</sub> directly. The reduction step could be achieved if it were coupled to ATP hydrolysis; however, HypD has no obvious ATP-binding sites. Our data revealed that HypD indeed catalyses ATP hydrolysis. Using an HPLC-based ATPase assay, we could show that the HybG-HypD and HypC-HypD complexes have a 5- to 10-fold higher ATPase activity than the intrinsic activity of these Hyp proteins alone [3]. The conserved Cys41 residue of HypD is essential for the ATPase-activity and is suggested to be important for an ATP-dependent conformational activation of HypD. Using limited proteolysis, we could show that HypD undergoes a conformational change when it binds ATP. In addition, the Cys41 residue of HypD appears to be involved in the conformational change induced by ATP because, while wildtype HypD without bound ATP is resistant to trypsin digestion, a HypD<sub>C41A</sub> variant is sensitive to trypsin. Together, our results suggest that HypD undergoes an ATP-dependent conformational activation step that facilitates binding of HybG or HypC and subsequent activation of ATP hydrolysis, which is proposed to be necessary for substrate reduction.

[1] Soboh, B. *et al.*, *Biochem. J.* **2014**, 464, 169-177.

[2] Adamson H. *et al.* *Anal. Chem.* **2017**, 89, 1565-1573.

[3] Nutschan, K. *et al.* *FEBS Open Bio.* **2019**, in press.

### 139-EPAP

Biochemical insights into the electron confurcating lactate dehydrogenase of *Acetobacterium woodii*

\*A. Katsyv<sup>1</sup>, V. Müller<sup>1</sup>

<sup>1</sup>Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt, Germany

**Introduction:** Flavin-based electron bifurcation is a novel mechanism to couple endergonic and exergonic redox-reactions widespread in anaerobes. However, knowledge about the mechanism of coupling is still limited. The acetogenic model organism *Acetobacterium woodii* has three different bifurcating enzymes: the hydrogenase, the caffeoyl-CoA reductase and the lactate dehydrogenase. The LDH of *A. woodii* forms a stable complex with two electron-transfer flavoproteins (Etf) and uses flavin-based electron



confurcation to drive endergonic lactate oxidation according to: lactate +  $\text{Fd}^{2+}$  + 2  $\text{NAD}^+$   $\rightarrow$  pyruvate +  $\text{Fd}$  + 2  $\text{NADH}$ .

**Objectives:** We aimed to elucidate the molecular basis of electron flow in the electron-confurcating lactate dehydrogenase reaction.

**Materials & methods:** For creating site-directed enzyme variants, we established a heterologous production system. The encoding LDH/Etf genes from *A. woodii* fused with a Strep-tag were cloned into a pET-based expression vector and expressed in *Escherichia coli* BL21(DE3) $\Delta$ iscR. The LDH/Etf complex was purified under anoxic conditions by Strep-Tactin affinity and size exclusion chromatography. Activity of the variants was analysed spectrophotometrically.

**Results:** The confurcating LDH/Etf complex was purified to apparent homogeneity and catalyzed electron-confurcation from reduced ferredoxin and lactate to  $\text{NAD}^+$  with a specific activity of 14,5 U/mg. The bifurcation reaction could be observed with 1,2 U/mg. Certain variants with alterations in  $\beta$ -flavin- or  $\text{NAD}^+$ -binding site as well as the possible electron transfer pathway were generated. These mutations all abolished confurcation/bifurcation up to 85-90%. The deletion of Fe/S-cluster and of the whole Fe/S-domain even resulted in a complete loss of confurcation ability but reduced bifurcation only by 80%.

**Conclusion:** Key amino acids, coordinating catalytic centers like FAD,  $\text{NAD}^+$  or Fe/S and the possible electron transfer pathway, were identified and shown to have an impact on the confurcational/bifurcational mechanism. For the first time, evidence is present that especially the Fe/S-domain is indeed essential for the confurcation reaction.

*G. sulfurreducens* was cultivated in 100 mL single and double chamber batch reactors with 5 mM acetate using a transparent conductive material as working electrode (glass sputter-coated with 10 nm of Cr and 25 nm of Au/Pd 80/20 %), a graphite rod as counter electrode, and an Ag/AgCl sat. KCl (+197 mV vs. SHE) as reference electrode. In addition to mechanistic electrochemical analysis (cyclic voltammetry), HPLC samples were taken and the biofilm was imaged with CLSM. The biomass dry weight was quantified and related to the volume of one single cell assuming rod shape (1.5  $\mu\text{m}$  length and 0.6  $\mu\text{m}$  width)<sup>1</sup>.

### Results:

For single chamber systems, it was demonstrated that the applied potential did not influence the coulombic efficiency, but the growth kinetics. Further, cell density within biofilm and the formal potential of the EET redox centers were constant. The yield coefficient of the biofilm grown at +0.2 V was lower (2-3 pmol  $\text{e}^-$  per replication) compared to -0.2 V and 0 V (0.2-0.6 pmol  $\text{e}^-$  per replication).

### Conclusion:

We demonstrated that the potential has a strong impact on biomass yield per electron. The lower yields at more positive potentials indicate a metabolic shift of the growth of *G. sulfurreducens* from effectivity to speed, as also supported by modelling<sup>2</sup>.

<sup>1</sup>ACS Nano 2019, 23, 4834

<sup>2</sup>Bioelectrochemistry 2015, 106, 194

## 140-EPAP

Determination of yield coefficients of electroactive microorganisms – example of *Geobacter sulfurreducens*

\*F. Scarabotti<sup>1</sup>, B. Korth<sup>1</sup>, M. Schmidt<sup>2</sup>, L. Rago<sup>1</sup>, K. Bühler<sup>3</sup>, F. Harnisch<sup>1</sup>

<sup>1</sup>Helmholtz-Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Helmholtz-Centre for Environmental Research - UFZ, ProVIS - Centre for Chemical Microscopy, Dept. Isotope Biogeochemistry, Leipzig, Germany

<sup>3</sup>Helmholtz-Centre for Environmental Research - UFZ, Department Solar Materials, Leipzig, Germany

### Introduction:

Electroactive microorganisms (EAM), e.g. *Geobacteraceae*, are able to use solid terminal electron acceptors like metals or anodes. This is known as extracellular electron transfer (EET) and can be exploited in primary microbial electrochemical technologies. However, although several details of the molecular mechanisms of EET are deciphered, there is a significant lack of quantitative information on EAM.

### Objectives:

Due to the lack of knowledge about yield coefficients and kinetic data of EAM proliferation, it was the aim of this study to reveal these on the (averaged) single cell level. Therefore, the (averaged) charge per replication per single cell of the model organism *Geobacter sulfurreducens*, biomass, cell density, and the influence of the anode potential was analyzed.

### Materials & methods:

## 141-EPAP

Degradation of the sugar alternative 5-keto-D-fructose by the human intestinal microbiota

\*J. Schiessl<sup>1</sup>, K. Kosciow<sup>1</sup>, U. Deppenmeier<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität, Institut für Mikrobiologie und Biotechnologie, Bonn, Germany

Obesity and diabetes belong to the major health problems of today's society. High-calorie food and the excessive consumption of sugars contribute to the development of these diseases. It became evident that the elevated intake of these compounds leads to an altered microbiota composition in the human gut. Hence, the analysis of the physiology and the growth requirements of the corresponding bacteria is of major interest. One aim of this study was to examine the degradation of the potential low-calorie sugar substitute 5-keto-D-fructose (5-KF). We identified different 5-KF reductases that are able to reduce 5-KF to D-fructose or L-sorbose, which are channelled into the central catabolic pathways of the corresponding microorganisms. Such enzymes were found in *Tatumella morbirosei* and *Gluconobacter oxydans*, which do not belong to the intestinal microbiota. Homologous proteins were also detected in the anaerobic soil bacterium *Clostridium pasteurianum*. For a detailed characterization the ketofructose reductase (KFR) of *T. morbirosei*, the enzymes Gox0644 and Gox1432 of *G. oxydans* and an aldoketoreductase of *C. pasteurianum* were heterologously produced in *E. coli* and purified. The highest activity was detected for the KFR from *T. morbirosei* (1428  $\pm$  82,8 U/mg protein). In addition, the substrate spectrum of the enzymes was investigated. The KFR of *T. morbirosei* specifically reduced 5-KF to D-fructose and can be used as a sensitive tool to quantify even very low 5-KF concentrations in different foods. All other enzymes reduced 5-KF to L-sorbose. These enzymes had a broad substrate spectrum

and reduced different ketones, aldehydes and sugars. Although highly similar enzymes are encoded by most of the intestinal microorganisms, none was able to metabolize 5-KF.

---

#### 142-EPAP

Phthalate degradation via phthaloyl-CoA in anaerobic and aerobic bacteria

\*R. A. Geiger<sup>1</sup>, M. Boll<sup>1</sup>

<sup>1</sup>University of Freiburg, Faculty of Biology, Freiburg im Breisgau, Germany

The environmentally relevant xenobiotic phthalic acid esters (PAEs) are annually produced at the million ton scale and are mainly used as plasticizers that are non-covalently incorporated into high-molecular weight polymers such as polyvinyl chloride<sup>1</sup>. After migration of PAEs into the environment, microbial hydrolysis via esterases into respective alcohols and phthalates occurs under aerobic and anaerobic conditions. Until recently, aerobic PA degradation only was associated with the introduction of hydroxyl functionalities by dioxygenases to facilitate the further decarboxylation step to protocatechuate via cofactor-free decarboxylases<sup>1</sup>. In contrast, denitrifying and sulfate-reducing bacteria activate PA to the possibly most instable CoA ester present in nature – phthaloyl-CoA (half-life ~7 min), by either ATP-dependent CoA ligases or CoA transferases, followed by decarboxylation to the central intermediate benzoyl-CoA via ubiD-like phthaloyl-CoA decarboxylase (PCD)<sup>2</sup>. Surprisingly, we identified three denitrifying PA degraders use a hybrid pathway for aerobic PA degradation including the activation and decarboxylation of PA to benzoyl-CoA. We present the properties of phthaloyl-CoA decarboxylase that accomplishes decarboxylation at a prenylated FMN cofactor that depends on Fe<sup>2+</sup> and K<sup>+</sup> ions.

<sup>1</sup>Boll M, Geiger RA, Junghare M, Schink B (2019). Microbial degradation of phthalates: biochemistry and environmental implications. *Environmental microbiology reports*, doi: 10.1111/1758-2229.12787

<sup>2</sup>Geiger RA, Junghare M, Mergelsberg M, Ebenau-Jehle C, Jesenofsky VJ, Jehmlich N, von Bergen M, Schink B, Boll M (2019). Enzymes involved in phthalate degradation in sulphate-reducing bacteria. *Environmental microbiology*, (10):3601-3612

---

#### 143-EPAP

The microbial conversion of estrogens into androgens via a SAM-, ATP- and Co-dependent methyl transfer

\*J. Krull<sup>1</sup>, C. Jacoby<sup>1</sup>, M. Boll<sup>1</sup>

<sup>1</sup>Faculty of Biology, Freiburg, Germany

Estrogens such as 17 $\beta$ -estradiol (E2) or estrone (E1) are biologically active hormones and their release to the environment causes endocrine disruption in wildlife. Estrogens are biosynthesized from cholesterol and their formation from androgens is catalyzed by cytochrome P450 aromatases. Estrogen biosynthesis is exclusively found in eukaryotes, but only bacteria are capable of using estrogens as sole carbon and energy source. Aerobic bacteria use the O<sub>2</sub>-dependent 4,5-seco pathway to activate and break down the aromatic A-Ring of estrogens<sup>1</sup>. However, the genes and enzymes involved in denitrifying estrogen degraders have remained elusive. Recently, an E2-induced gene cluster

*emtABCD* was identified in *Denitratisoma* sp. strain DHT3 that encodes a putative Co-methyltransferase<sup>2</sup>. Proteome analysis in *Denitratisoma oestradiolicum* clearly identified next to *emtABCD* also genes involved in SAM recycling suggesting a cobalamin-dependent methyltransferase that uses SAM as methyl donor. In vitro assays revealed the SAM- and ATP-dependent methyl transfer to estrogen resulting in the dearomatization of ring A to 1-dehydrotestosterone.

<sup>1</sup>Chen Y-L, et al. (2017). Biochemical Mechanisms and Catabolic Enzymes Involved in Bacterial Estrogen Degradation Pathways. *Cell Chemical Biology* 24.

<sup>2</sup>Wang P-H, et al. (2019). Retroconversion of estrogens into androgens by bacteria via a cobalamin-mediated methylation. *BioRxiv* preprint. <http://dx.doi.org/10.1101/714501>

---

#### 144-EPAP

Three enzymes involved in enzymatic hydroxylation of a primary carbon with water during anaerobic cholesterol degradation

\*C. Jacoby<sup>1</sup>, M. Boll<sup>1</sup>

<sup>1</sup>University of Freiburg, Faculty of Biology, Freiburg im Breisgau, Germany

The functionalization of C-H-bonds represents a challenging enzymatic reaction and is accomplished by oxygenases in aerobic organisms. In anaerobic bacteria such reactions are no option and a number of unprecedented enzymatic strategies have been identified for O<sub>2</sub>-independent C-H-bond activation. In the denitrifying cholesterol degrading *Sterolibacterium denitrificans* the primary C-26 is oxidized to a carboxylic acid in the absence of oxygen. Surprisingly hydroxylation takes place at the tertiary C-25 by a Mo-dependent Steroid C25 hydroxylase (S25DH) using water as hydroxyl-donor<sup>1,2</sup>. We identified that the tertiary alcohol is dehydrated in an ATP-dependent manner, yielding an allylic alkene product. This dehydration is essential for the subsequent hydroxylation of the terminal C26 carbon atom via an allylic cation intermediate. The C26 hydroxylation and further oxidation to an aldehyde is catalyzed by a bifunctional S25DH-like Mo-containing enzyme (S26DH) that is indispensable for the consecutive degradation of the steroidal side chain by  $\beta$ -oxidation like reactions. Remarkably, oxidation of the primary carbon takes place by alternating periplasmic and cytoplasmic enzymatic reactions.

<sup>1</sup>Dermer J and Fuchs G (2012). Molybdoenzyme that catalyzes the anaerobic hydroxylation of tertiary carbon atom in the side chain of cholesterol. *J Biol Chem* 287.

<sup>2</sup>Jacoby C, et al. (2018). Four Molybdenum-Dependent Steroid C25 Hydroxylases: Heterologous Overproduction, Role in Steroid Degradation, and Application for 25-Hydroxyvitamin D3 Synthesis. *mbio* 9.

---

#### 145-EPAP

Contrasting Apparent Michaelis-Menten Constants of Nitrate/Nitrite Reduction in Cell Suspensions of Denitrifiers and Nitrate Ammonifiers

\*D. Frohloff<sup>1</sup>, M. A. Horn<sup>1</sup>

<sup>1</sup>Leibniz Universität Hannover, Institut für Mikrobiologie, Hannover, Germany

Denitrification is an anaerobic respiratory process where nitrate and/or nitrite serve as electron acceptors and are converted to gaseous products like  $\text{N}_2\text{O}$  and/or  $\text{N}_2$  via several intermediates. This process is favored at high nitrate concentration and a low C/N ratio relative to the dissimilatory nitrate reduction to ammonium (DNRA). DNRA is a competing process for nitrate reduction performed by nitrate ammonifiers and is favored at low nitrate concentration and a high C/N ratio. Contrasting kinetics for electron acceptor usage for denitrifiers and nitrate ammonifiers might help to explain their competitiveness for nitrate and nitrite. However, few studies to date have addressed such kinetic parameters for nitrate and nitrite reduction (apparent Michaelis-Menten kinetics, maximum uptake rate  $V_{\max}$  and half-saturation constant  $K_m$ ). Thus, the objective of this study was to determine apparent Michaelis-Menten parameters for nitrate and nitrite with intact cells of denitrifiers, nitrate ammonifiers and bacteria capable of both processes. Selected bacterial strains of five denitrifiers, one DNRA and one strain that is capable of both processes were grown under anoxic conditions with nitrate as an electron acceptor until the late exponential phase. After several washing steps and resuspension in modified medium, the culture was spiked with different nitrate/nitrite concentrations ranging from 5 – 600  $\mu\text{M}$ . Subsequently, the rates of nitrate/nitrite reduction were determined for each concentration. The apparent  $K_m$  were in the range of 10 – 43  $\mu\text{M}$  ( $\text{NO}_3^-$ ) and approximated 15  $\mu\text{M}$  ( $\text{NO}_2^-$ ) for denitrifiers. In contrast, the apparent  $K_m$  for DNRA were approximately 3  $\mu\text{M}$  for  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . Consequently, the data suggest a higher affinity of DNRA for nitrate and nitrite relative to denitrifiers, which helps to explain the competitive advantage of DNRA under low nitrate/ nitrite conditions.

#### 146-EPAP

Illuminating the function of the hydrogen-dependent  $\text{CO}_2$ -reductase

\*H. M. Dietrich<sup>1</sup>, V. Müller<sup>1</sup>

<sup>1</sup>Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt, Germany

**Introduction:** Acetogenic bacteria utilize the Wood-Ljungdahl pathway for  $\text{CO}_2$  fixation and energy conservation. In some of these bacteria, as in *Thermoanaerobacter kivui*, the initial step in  $\text{CO}_2$  reduction is catalyzed by a hydrogen-dependent  $\text{CO}_2$ -reductase (HDCR). This enzyme complex consists of a hydrogenase (HydA2), a formate dehydrogenase (FdhF), and two putative electron-transferring subunits (HycB3 & HycB4). The HDCR reduces  $\text{CO}_2$  to formate, using molecular hydrogen as direct electron donor but is also able to perform the reverse reaction with the same efficiency. This highlights the HDCR as an auspicious candidate for a bio-based system for hydrogen storage as well as formate production. The operating principle of the HDCR is yet to be understood. To pave the way for the development of a competitive biotechnological process, specific knowledge of the enzymatic reactions is needed.

**Objectives:** We aimed to unravel the structure, molecular function and electron flow in the hydrogen-dependent  $\text{CO}_2$ -reductase.

**Materials & methods:** For generating site-directed mutagenesis- and deletion variants of the HDCR, we established a homologous overproduction system. The genes for the *hdcr* operon variants were cloned into an expression vector and transformed into a *T. kivui*  $\Delta hdcr$  strain. The HDCR derivatives were purified by affinity chromatography and activity of the variants was analyzed.

**Results:** The *T. kivui* HDCR complex was overproduced homologously in *T. kivui*, resulting in a strain showing a 35-fold increase in HDCR activity. The HDCR complex was purified in a single step and catalyzed its specific reaction in both directions with the highest rates shown to date. Different enzyme variants with alterations in its subunit composition as well as modifications in the active sites or Fe/S-clusters were created to reveal the organization of the complex as well as the reaction mechanism during the HDCR reaction.

**Conclusion:** Deletion variants reveal HycB3 and HycB4 as central electron transferring proteins flanked by the catalytic HydA2 and FdhF. Identifying essential Fe/S-clusters and amino acids coordinating the active centers allowed us to create a 3D-model of the HDCR.

---

#### 147-EPAP

**Antimicrobial resistance: the interplay of biocides and antibiotics affecting resistance evolution in bacteria**

\*F. Schreiber<sup>1</sup>, S. Schmidt<sup>1</sup>, N. Nordholt<sup>1</sup>, F. Pietsch<sup>1</sup>, G. Heinrich<sup>1</sup>

<sup>1</sup>Federal Institute for Materials Research and Testing (BAM), Materials and Environment, Berlin, Germany

Biocides are important to ensure hygiene in sensitive settings like hospitals and to protect materials used in industry and buildings. Therefore, in many applications biocides are used near to humans and the microbiomes of human-inhabited environments are exposed to biocides. An important issue related to the use of biocides is that bacteria can evolve resistance to biocides and that the evolved mechanisms can confer cross-resistance to antibiotics. In this presentation, I will discuss the evolutionary mechanisms that affect resistance evolution by the interplay of biocides and antibiotics. Furthermore, I will highlight examples of our current work on biocide resistance evolution with an emphasis on 3 aspects: (i) effects of biocides on basic evolutionary drivers for resistance including mutation rates and horizontal gene transfer, (ii) evolution of persistence to biocides as a survival strategy on the single-cell level, (iii) synergy and antagonism induced by biocide-antibiotic combinations. We show that biocides increase mutation rates but not horizontal gene transfer by conjugation. In addition, persistence is a potential survival strategy for bacteria against biocides; especially against those that are membrane active. Specific biocide-antibiotic combinations display strong antagonistic or synergistic activities leading to potential selection effects. I will further present molecular data that explains the underlying mechanisms of these phenomena.

---

#### 148-EPAP

**Moderated Evaluation of Anaerobic Ammonium Oxidizing (Anammox) Bacteria Activities during Anoxic Treatment Work Flows**

\*E. Ude<sup>1</sup>, C. Ding<sup>2</sup>, L. Adrian<sup>3,4</sup>

<sup>1</sup>UFZ, ISOBIO, Leipzig, Germany

<sup>2</sup>UFZ, ISOBIO, LEI, Germany

<sup>3</sup>Technische Universität Berlin, Berlin, Biotechnology, Berlin, Germany

<sup>4</sup> Technische Universität Berlin, Berlin, Germany, Geobiotechnology, Berlin, Germany

The Anammox process has been put forward as a new and promising way to treat wastewater containing high ammonium concentration using nitrite as electron acceptor. However, the implementation has been limited by various challenges. In spite of strong efforts, obtaining a pure culture of anammox bacteria has not been possible. The process is not fully understood in its interactions with different

compounds such as reductant, organic solvents, and antibiotics that are found potentially in animal waste or wastewater. Here, we hypothesize that understanding the metabolic and physiological activities of anammox bacteria within short term and long term period in various conditions is a key strategy to overcome the limitations in its implementations. In this study, series of batch reactors containing synthetic medium, effectors and inoculum were set for anammox cultivation and enrichment. Nitrite reduction, ammonium oxidation, nitrogen gas production, and heme production were monitored. Proteomic analysis and mathematical modelling were performed. Our results shows that at low cell densities, the rate of metabolism and activities of anammox bacteria declined compared to higher cell densities. Much of this inhibitors effect is due to density-dependent reductions in the effective concentration of inhibitors. Therefore, moderated inoculum and effectors are key strategy for anammox activities. Our results can help to determine favorable conditions for implementation of the process in large scale.

Keyword: Anammox, growth, metabolism, effectors, activities

## 149-EPAP

### Thermal Proteome Profiling—A High-Throughput Screening of the Proteome for Interactions with Substrates

\*D. Türkowsky<sup>1</sup>, P. Lohmann<sup>1</sup>, M. Mühlenbrink<sup>1</sup>, T. Schubert<sup>2</sup>, L. Adrian<sup>3</sup>, T. Goris<sup>2</sup>, N. Jehmlich<sup>1</sup>, M. von Bergen<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Molecular Systems Biology (MOLSYB), Leipzig, Germany

<sup>2</sup>Friedrich Schiller University, Institute of Microbiology, Department of Applied and Ecological Microbiology, Jena, Germany

<sup>3</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Isotope Biogeochemistry, Leipzig, Germany

In anaerobic bacteria, identification of protein-substrate interactions is a fundamental challenge. Thermal proteome profiling (TPP) is increasingly applied in eukaryotes. This technique allows assessing protein-ligand binding by quantifying protein melting curve shifts induced by the presence of a ligand.

We applied TPP to *Sulfurospirillum multivorans*, a bacterium which uses trichloroethene (TCE), a widespread groundwater pollutant, as electron acceptor for growth. Thereby, the interaction of TCE with the proteome of *S. multivorans* was investigated. We extended the protein detection range and facilitated the investigation of oxygen-sensitive bacterial proteins by several modifications of the original protocol (e.g., incubation under anaerobic conditions or increasing the temperature range up to 97°C). Omitting the electron donor during incubations allowed preventing enzymatic reductive dehalogenation.

As a result, we detected the interaction of TCE and the key enzyme of *S. multivorans*, the tetrachloroethene reductive dehalogenase PceA. As TCE is a known substrate of this enzyme, this confirmed the suitability of the method. Interestingly, a putative response regulator as part of a two-component regulatory system showed a similar trend as PceA. This is the first biochemical hint for its suggested role in TCE respiration.

We proved that our TPP approach serves to identify protein-substrate interactions of strictly anaerobic reductive dehalogenases and probably their regulators. This strategy can be used for the proteome-wide identification of proteins binding to other chemicals or even endogenous metabolites. It has the potential to be applied to bacteria which are difficult to investigate, for which only low amounts of biomass are

available or which are part of complex microbial communities.

Keywords: Protein interaction; Mass spectrometry; Organohalide respiration; Quantitative Proteomics

## 150-EPAP

Carbon source dependent reprogramming of anaerobic metabolism in *Staphylococcus aureus*

A. Giese<sup>1</sup>, K. Methling<sup>2</sup>, D. Zühlke<sup>1</sup>, M. Lalk<sup>2</sup>, G. Bange<sup>3</sup>, K. Riedel<sup>1</sup>, J. Bernhardt<sup>1</sup>, \*J. Pant-Farre<sup>1</sup>

<sup>1</sup>Universität Greifswald, Mikrobiologie, Greifswald, Germany

<sup>2</sup>Universität Greifswald, Biochemie, Greifswald, Germany

<sup>3</sup>Universität Marburg, Synmikro, Marburg, Germany

To be a successful pathogen, *S. aureus* has evolved a large spectrum of virulence strategies to counteract the highly effective innate and adaptive immune response of its host. However, of equal importance to establish an infection is the ability of *S. aureus* to adapt its metabolism to the host environment. For instance, inactivity of the respiratory chain, e.g. caused by the destructive action of nitric oxide radicals released by activated immune cells, will tie *S. aureus* survival to its capacity to produce energy by substrate level phosphorylation and mixed acid fermentation.

Using a combination of proteome, transcription and metabolome analyses, we show that fermentation pathways are fine-tuned in *S. aureus* in response to the available carbon source. In the absence an energy source that can be metabolized via glycolysis, the available carbon sources are channeled towards the production of Acetyl-CoA in a pyruvate formate-lyase (Pfl) dependent reaction and ATP is generated by the concerted activity of phosphate acetyltransferase (Pta) and acetate kinase (AckA). In parallel, expression of pathways that would divert the Acetyl-CoA from Pfl are downregulated. This switch in substrate channeling is controlled by the Catabolite control protein A (CcpA). The high flexibility in the adaption of the *S. aureus* anaerobic metabolism further highlights the importance of anaerobiosis as effective survival strategy in low oxygen environments.

## 151-EPAP

Structural Insights Into ATP-dependent Enzymatic Benzene Ring Reduction

\*J. Fuchs<sup>1,2</sup>, O. Tiedt<sup>1</sup>, U. Demmer<sup>3</sup>, U. Ermler<sup>3</sup>, M. Boll<sup>1,2</sup>

<sup>1</sup>Institute of Biology II / University of Freiburg, Microbiology, Freiburg i. Br., Germany

<sup>2</sup>University of Freiburg, RTG1976 - Cofactors, Freiburg i. Br., Germany

<sup>3</sup>Max-Planck-Institute for Biophysics, Molecular Membrane Biology, Frankfurt a. M., Germany

Anaerobic bacterial degradation of monoaromatic compounds proceeds via the central intermediate benzoyl-CoA (BCoA), catabolized by dearomatizing benzoyl-CoA reductases (BCRs). Class I BCRs couple the reduction of the substrate to cyclohexa-1,5-diene-1-carboxyl-CoA to a stoichiometric ATP hydrolysis to ADP and P<sub>i</sub><sup>1</sup>. A "Birch-like" reaction mechanism via radical intermediates was proposed to achieve substrate reduction at E<sup>0'</sup> = -622 mV, one of the most negative redox potentials of a redox couple in biology<sup>2</sup>.

During studies on 3-methylbenzoate degradation in several denitrifying bacteria, a class I BCR was identified with an unprecedented wide substrate spectrum including methylated and chlorinated substrates<sup>3</sup>. We succeeded in

heterologous production of the enzyme in *Escherichia coli* and solved the crystal structure of the catalytic subunits at 1.8 Å. The result provides evidence for the proposed radical-based mechanism at an active site [4Fe-4S]-cluster.

1. Buckel *et al.*, 2014. *Chem.Bio.Chem.* 15, 2188–2194
2. Kung *et al.*, 2010. *J.Am.Chem.Soc.* 132, 9850-9856
3. Tiedt *et al.*, 2018. *J.Biol.Chem.* 293(26), 10264-10274

## 152-EPAP

### Glutamate fermentation in Enterobacteria

\*J. Eggers<sup>1</sup>, I. Berg<sup>1</sup>

<sup>1</sup>Institut für Molekulare Mikrobiologie und Biotechnologie, WWU Münster, Münster, Germany

The methylaspartate pathway is one of five known pathways for bacterial fermentation of glutamate. In this pathway, glutamate is isomerised to methylaspartate, which is further deaminated to mesaconate, hydrated to (S)-citramalate, and the latter compound is finally cleaved to acetate and pyruvate. In our former studies [1] we have shown that the genomes of most of the *Escherichia coli* O157:H7 strains possess a cluster coding for the key enzymes of the methylaspartate pathway. Nevertheless, our attempts to detect the activities of corresponding enzymes in cell extracts of *E. coli* O157:H7 strain ATCC 700728 failed, and this strain could not ferment glutamate [1]. However, this strain is classified to Biosafety level 2 and might be deficient in some pathogenicity-related features in comparison to other enterohemorrhagic *E. coli* (EHEC) strains (Biosafety level 3). Here we decided to study another strain of enterobacteria, *Raoultella planticola* JCM 20069, in which the activity of methylaspartase was measured previously [2]. The genome sequencing of this strain showed the presence of a gene cluster similar to the glutamate fermentation cluster found in the EHEC strains. Furthermore, the studied strain was able to ferment glutamate, and we were able to detect activities of several key enzymes of the pathway during anaerobic growth on glutamate. We conclude that this pathway is used by *R. planticola* and most probably by EHEC strains to ferment glutamate. The presence of the methylaspartate cycle genes in EHEC strains suggests that glutamate and glutamine present in intestinal tract serve as energy sources, promoting pathogenicity.

[1] M. Kronen and I. Berg, *PLOSone* **12**, 1-18 (2015).

[2] Y. Kato and Y. Asano, *Arch Microbiol* **168**,457-463 (1997).

## 153-EPAP

### Proteogenomic analysis of the denitrifying betaproteobacterium *Aromatoleum aromaticum* pCyN1

\*P. Becker<sup>1</sup>, A. Strijkstra<sup>1</sup>, L. Wöhlbrand<sup>1</sup>, R. Reinhardt<sup>2</sup>, R. Rabus<sup>1</sup>

<sup>1</sup>Carl von Ossietzky Universität Oldenburg, Institut für Chemie und Biologie des Meeres (ICBM), Oldenburg, Germany

<sup>2</sup>Max Planck-Genome-centre Cologne, Cologne, Germany

## Introduction

*Aromatoleum aromaticum* pCyN1 is a facultative denitrifying betaproteobacterium capable of anaerobic degradation of a wide variety of monoaromatic compounds and terpenes. *A. aromaticum* pCyN1 is a close relative of the model organism *A. aromaticum* EbN1.

## Objectives

The objectives of the present study were as follows: (i) determine the complete genome of strain pCyN1 and analyze it by manual annotation, (ii) perform differential proteomics across 30 different substrate adaptation conditions, (iii) integrate genomic and proteomic data to establish the catabolic network of strain pCyN1 and (iv) compare strains pCyN1 and EbN1 on the proteogenomic level.

## Materials & methods

Manual genome annotation and functional predictions were based on public non-redundant protein and protein domain databases. Analysis of the soluble and membrane protein-enriched fractions of substrate adapted cells of strain pCyN1 was performed by 2D DIGE and SDS-PAGE coupled to MS-based identification.

## Results

The genome of strain pCyN1 consists of a single circular 4.37 Mbp chromosome harboring 4,194 genes. Gene clusters for the anaerobic degradation modules are scattered across the genome. The elucidated catabolic network comprises of 112 proteins, 104 of which have been identified. Individual reaction sequences are to the most part regulated with high substrate-specificity. Notably, strain pCyN1 possesses three parallel central pathways: (i) the classical benzoyl-CoA pathway serving 16 aromatic compounds, (ii) a terpene (6 different)-specific benzoyl-CoA reductase in conjunction with a likewise specific modified  $\beta$ -oxidation, and (iii) a third  $\beta$ -oxidation sequence for the degradation of cyclohexanecarboxylate. Comparison with *A. aromaticum* EbN1 underlined high plasticity of the genomes of both species, and revealed specific genomic regions for the characteristic substrates *p*-cymene (pCyN1) and ethylbenzene (EbN1).

## Conclusion

The present study furthers our understanding of the catabolic potential harbored in degradation specialists of the new genus *Aromatoleum*.

## 154-EPAP

### Lactate-based microbial chain elongation for *n*-caproate production: genomic and metabolic features of three novel *Clostridiales* isolates

\*B. Liu<sup>1</sup>, D. Popp<sup>1</sup>, H. Sträuber<sup>1</sup>, H. Harms<sup>1</sup>, S. Kleinsteuber<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany

Caproate (C6) is a valuable product of the carboxylate platform that can be used, for instance, as precursors for the production of antimicrobials and drop-in biofuels. The cyclic reverse  $\beta$ -oxidation pathway, also known as microbial chain elongation (CE), is a key metabolic function to produce C6. Hitherto, only few species have been reported for the conversion of lactate to C6. Here we describe three novel bacterial strains that were isolated from an anaerobic bioreactor producing medium-chain carboxylates from maize silage. They can produce C6 and *n*-butyrate using lactate as sole carbon source. Two of them can additionally produce *i*-butyrate in different proportions. All three strains showed low sequence similarity to previously described species based on whole 16S rRNA gene sequencing analysis. They share the highest similarities with *Clostridium luticellarii* (97.34%), *C.*

*jeddahense* (96.25%) and *Ruminococcaceae* bacterium CPB6 (94.06%), respectively. We sequenced the whole genomes using the Oxford Nanopore and Illumina Miseq platforms. Three circular genomes were constructed using a hybrid assembly approach. Based on the high-quality genome results, one strain was affiliated to the genus *Clostridium* and contains one circular chromosome of 3.86 Mbp with a GC content of 34.32% and one circular 55 kb plasmid. In total, 3867 protein-encoding sequences and 5 rRNA operons were identified. The other two strains affiliated to the *Clostridiales* contain circular chromosomes of 2.34 Mbp with a GC content of 42.75% and 3.44 Mbp with a GC content of 54.63%, respectively. 2319 and 3480 protein-encoding sequences, respectively, and 3 rRNA operons in each strain were identified. Genes involved in acetyl-CoA formation from lactate, reverse  $\beta$ -oxidation and energy conservation by flavin-based electron bifurcation and ferredoxin:NAD oxidoreductase (Rnf complex) were identified. Complete pathways for CE with lactate via CoA transferase and thioesterase were reconstructed. The results provide a genome-centered rationale to understand the metabolism for the C6 production from lactate. Detailed mechanisms involved in the formation of C6 and *t*-butyrate need to be further studied.

### 155-BTP

Phytochemical screening and GC-MS analyses of ethanoic leaves extracts of *Mitracarpus scaber*

\*F. O. Ekhaie<sup>1</sup>

<sup>1</sup>University of Benin, Department of Microbiology, Faculty of Life Sciences, Benin City, Nigeria

*Mitracarpus scaber* is a widely used plant in traditional medicine practice in West Africa. This study was designed to investigate the bioactive components of the ethanolic leaves extracts of *Mitracarpus scaber* using Gas Chromatography – Mass Spectrometry (GC - MS) analysis. The GC-MS analysis of the ethanolic leaves extract of *M. scaber* was performed on a GC-MS equipment (GC-MS QP 2010 plus fused with a GC column (AOC 2i)) coated with polymethyl silicon with a dimension of 0.25mm X 30mm. The GC-MS analysis revealed the presence of 19 bioactive components of *M. scaber*. The bioactive components present in the ethanolic extract include 5-Nanonol (29.71%), 5-hydroxy-2-(hydroxyl methyl)-4H-pyran-4-one (24.95%), 2-octenoic acid (0.34%), 1 -hydroxy-2,2,6,6-tetramethyl-3-(4-nitroso-1(piperazinylmethyl) – piperidin-4-onr (12.72%), Pentadecanoic acid (6.37%), 1, 2-epoxyhexadecane (0.07%), Phytol (2.53%), 6- octadecenoic acid (7.23%), 1, 2-Benzenedicarboxylic acid (8.60%) and 11- bromoundecanoic acid (1.10%). The results of the study revealed that *Mitracarpus scaber* leaves possesses antimicrobial effects. The presence of these components in the plants extract are known to be responsible for the pharmacological properties of *M. scaber*, as it is recommended as a plant of phytotherapeutics importance.

Keywords: GC-MS, *Mitracarpus scaber*, leaves, ethanol and bioactive compounds.

### 156-BTP

Electrode-driven anaerobic production of chemicals using *Pseudomonas putida*

\*B. Lai<sup>1</sup>, A. V. Nguyen<sup>1,2</sup>, \*J. Krömer<sup>1,2</sup>

<sup>1</sup>Helmholtz Center for Environmental Research - UFZ, Solar Materials, Leipzig, Germany

<sup>2</sup>Helmholtz Center for Environmental Research - UFZ, Leipzig, Germany

*Pseudomonas putida* (*P. putida*) can be a promising industrial chasis for biochemical production because of its versatile cellular metabolism. While many limiting factors needs to be addressed for its industriliation, one of them will be its solely dependency of cellular metabolism on oxygen, which makes the process engineering complicated and costly. In our group, we are trying to tackle this issue using electrochemical approach. By providing electrode as the electron acceptor, a high yield and robust biosynthesis process of chemicals based on electrode was established. Systems biology and metabolic engineering approaches were applied to reveal the molecular machnism for further strain and process developement.

The electron transfer route between *P. putida* and anode was established with the presence of redox mediator. A screening of different redox chemicals demonstrated effective mediator should have a minimum redox potential of 0.207V vs SHE. A further comparative proteomics analysis revealed the membrane respirotary components were all upregulated and combining with the electron inhibitors tests, cytochrome c reductase was demonstrated to be the protein interacting with mediator for extracellular electron transfer.

Driven by the electrode, a high yield and "redox-imbalanced" anoxic sugar acids production was subsequently developed. Over 90% of glucose was converted into 2-ketogluconate, a precursor for erythorbic acid production, in such a system. The production rate could be improved by 644%, if the glucose dehydrogenase was overexpressed. Other than glucose, this process was also applicable to other monosaccharides for specific unusual sugar acids production, including both growth and non-growth substrates for *P. putida*.

In summary, the first anaerobic process using an obligate aerobe was established by applying electrode as the final electron acceptor, and this unique platform can be developed into biosynthesis of value-added sugar acids from varied carbohydrates.

### 157-BTP

Styrene and indole degradation pathways provide access towards monooxygenases with reversed enantioselectivity

\*D. Tischler<sup>1</sup>, T. Heine<sup>2</sup>

<sup>1</sup>Ruhr-Universität Bochum, Microbial Biotechnology, Biology and Biotechnology, Bochum, Germany

<sup>2</sup>TU Bergakademie Freiberg, Environmental Microbiology, Freiberg, Germany

Styrene is a small aromatic compound mainly introduced to nature by anthropogenic activity while indole is a natural compound derived from plant material or produced during tryptophan metabolism. However, the microbial degradation pathways have many things in common. Especially, the first step is an enantioselective epoxidation by means of a flavin-dependent monooxygenase.

A phylogenetic study, production and biochemical validation of selected enzymes, and initial biotechnological investigations allowed describing this class of monooxygenases in more detail.

About 150 amino acid sequences of related styrene/indole monooxygenases have been analyzed with respect to sequence similarity, conserved motifs, adjacent genes, and host strains. More than 30 enzymes have been screened by means of recombinant production while 23 were obtained in an active form. All enzymes had in common that they produce solely (S)-styrene oxide from styrene and most likely

the same indole oxide from indole which is under debate. In all cases, the monooxygenases could be fueled by reduced FAD obtained either from a NADH-consuming flavin-reductase or an artificial reduction by an additional electron donor, 1-benzyl-1,4-dihydronicotinamide. Besides those natural substrates, it is known that flavin-dependent monooxygenases convert sulfides to chiral sulfoxides, which was evaluated for all enzymes obtained in this study. The indole monooxygenases preferably produced the (S)-enantiomer while styrene monooxygenases showed a tendency to produce the (R)-enantiomer of respective sulfoxides. This interesting finding was combined with our sequence analysis and a molecular docking approach of various substrates. Thus it was possible to identify two distinct fingerprint motives for the styrene (N46-V48-H50-Y73-H76-S96) and indole (S46-Q48-M50-V/I73-I76-A96) monooxygenases which allows to predict enantioselectivity in sulfoxidation reactions. Scale up experiments verified the applicability of these enzymes in biocatalysis.

Heine et al., ChemCatChem 2019, 11, 1–12

### 158-BTP

Fermentation and downstream processing in PILOT scale for the development of novel veterinary vaccines based on *Kluyveromyces lactis* yeast

\*M. Suhr<sup>1</sup>, N. Volk<sup>2</sup>, S. Bennewitz<sup>1</sup>, M. L. Hoffmann<sup>1</sup>, P. Franz<sup>1</sup>, H. Hennemann<sup>1</sup>, S. E. Behrens<sup>1,3</sup>

<sup>1</sup>VEROVACCINES GmbH, Halle/ Saale, Germany

<sup>2</sup>Martin Luther University Halle-Wittenberg, Faculty of Natural Sciences I - Life Sciences, Halle/ Saale, Germany

<sup>3</sup>Martin Luther University Halle-Wittenberg, Institute for Biochemistry and Biotechnology, Halle/ Saale, Germany

Our novel technology platform for veterinary vaccines is based on recombinant *K. lactis* expressing individual pathogen proteins which act as immunogens. These vaccines are highly effective, easily combinable and inexpensive to produce. *K. lactis* as a Crabtree-negative budding yeast shows low ethanol formation, high biomass and protein yields under high glucose concentration. The **gene of interest (GOI)** expression is regulated by the transcriptional activator KIGal4 and its repressor KIGal80. Multiple genetically engineered expression cassettes allow **multivalent vaccines** generation.

Therefore, a highly efficient, stable and reproducible fermentation and downstream process is needed that combines both a high biomass and GOI yield.

First, a transfer from laboratory scaled growth in shaking flasks using complex yeast media to a controlled **batch fermentation** (scale 1-8 L) using synthetic defined media was performed. By varying process parameters (e.g. temperature, pH, nutrients) we mapped growth characteristics.

After this, we established a **3-stage fed-batch** fermentation (**15 L pilot scale**) that are performed under the aspects of enhancing the biomass and GOI formation as well as on industrial economic point of views. The 1st step, a glucose batch fermentation for initial biomass formation is followed by subsequent glucose feeding to force biomass formation (2nd step). The 3rd step (induction) starts after a directed carbon source overlap with the inducer molecule. Depending of the GOI and its cytotoxic properties, the biomass formation is influenced and specific growth rates on lactose are lowered compared to growth rates on glucose. Finally, we established different exponential feeding strategies, initial biomass

concentration and induction times to obtain a highly efficient, stable and reproducible process.

The **downstream** process consists of biomass separation, freeze-drying, grinding and yeast **inactivation**. The **dried** and inactivated yeast powder is **thermostable** for longer periods and may be beneficial for the use in emerging and developing countries. The direct use of the complete material without any antigen purification is a simple and **inexpensive** process.

### 159-BTP

*Gluconobacter oxydans* strains for the production of the non-nutritive natural sweetener 5-ketofructose

\*K. Wohlers<sup>1</sup>, A. Kranz<sup>1</sup>, A. Wirtz<sup>1</sup>, M. Baumgart<sup>1</sup>, M. Bott<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, Institute for Bio- and Geosciences 1: Biotechnology, Jülich, Germany

**Introduction** 5-Ketofructose (5-KF) is considered as a promising natural low-calorie sweetener and due to the increasing demand for such compounds a highly interesting product. It can be formed by oxidation of fructose with the membrane-bound enzyme complex fructose dehydrogenase from *Gluconobacter japonicus*, which is encoded by the *fdhSCL* genes. In recent studies it was shown that plasmid-based overexpression of the *fdhSCL* genes in the biotechnologically established production host *Gluconobacter oxydans* allows efficient 5-KF production from fructose [1,2].

**Objectives** In order to generate a plasmid-free 5-KF-producing *G. oxydans*, we compared different genomic integration sites in the genome of the engineered *G. oxydans* strain IK003.1 [3] for their suitability to allow high-level *fdhSCL* expression.

**Materials & methods** Four chromosomal integration sites were selected and the *G. japonicus fdhSCL* gene cluster under control of the strong constitutive P264 promotor was integrated with pAJ63a-based plasmids [4]. Fructose and 5-KF concentrations of shake flask cultivations were determined via HPLC.

**Results** Integration of a single *fdhSCL* copy in three intergenic regions and in an inactive sorbitol dehydrogenase gene lead to four *G. oxydans* strains that slightly differed in the rates of 5-KF production, indicating position-specific effects. A strain with two genomic *fdhSCL* copies at the two most efficient integration sites was generated that showed two-fold increased 5-KF production rates, approaching plasmid-based 5-KF production, and yields of 0.73 g 5-KF per g fructose.

**Conclusion** A series of plasmid-independent *G. oxydans* strains capable of 5-KF production were generated, which will serve as basis for future strain development. Furthermore, four suitable chromosomal integration sites for heterologous gene expression were identified, which will be useful for further projects.

### References

[1] Siemen et al. (2018) Appl Microbiol Biotechnol 102 (4), 1699-710.

[2] Herweg et al. (2018) Biores Technol 259, 164–172

[3] Kiefler et al. (2017) Appl Microbiol Biotechnol 101 (13), 5453–67

[4] Peters et al. (2013) Appl Microbiol Biotechnol 97 (6), 2521–2530

## 160-BTP

*Vibrio natriegens* as host for biotransformation with a flavin-dependent monooxygenase

H. Fuchs<sup>1</sup>, \*T. Heine<sup>1</sup>

<sup>1</sup>TU Bergakademie Freiberg, Institute of Biosciences, Freiberg, Germany

## Introduction

Beyond *Escherichia coli*, various expression hosts have been investigated and are in use for biotechnological applications.<sup>[1]</sup> Therewith, several hosts are available for specific requirements of a biotechnological reaction setup. However, due to drawbacks of each individual system, there is continuous research on improvement of expression strains. Recently, the marine bacterium *Vibrio natriegens* gained interest, as it offers a remarkable fast growth rate.<sup>[2]</sup>

## Objectives

In this study, we aimed introducing a flavin-dependent group E monooxygenase into a modified *V. natriegens* (Vmax) for expression and biotransformation of aromatic compounds.

## Materials & methods

For transfer of the plasmid, the oriT from pK18mbosacB-tac<sub>le</sub>GFP was introduced into the pET16bP plasmid backbone, which also carried the gene encoding for a group E monooxygenase (*GnlIndA*). This construct was introduced into *E. coli* S17-1, which was applied as donor strain allowing for transfer of the vector into *V. natriegens* (Vmax). Success of the conjugation was tested by PCR and the monooxygenase was expressed and assayed as described previously.<sup>[3]</sup>

## Results

The generated expression construct was successfully transferred from *E. coli* into *V. natriegens* via conjugation. Expression of the monooxygenase was induced by IPTG and successful protein production was proven by SDS-Page. A whole cell biotransformation was performed using styrene as substrate, which was converted by the monooxygenase to styrene oxide. However, the product concentration decreased after 30 min indicating that the *Vibrio* is able to decompose the epoxide.

## Conclusion

*V. natriegens* is, i.a. due to the fast growth rate, a promising candidate for the expression of proteins as well as organism for biotransformations. This is the first study in which this strain was used for the conversion of aromatic compounds. However, further optimization and characterization is needed to establish *V. natriegens* as real alternative to other strains.

## References

[1] Gomes et al. (2016) Adv. Anim. Vet. Sci. 4:346–356.

[2] Weinstock et al. (2016) Nat. Methods 13:849–851.

[3] Heine et al. (2019) ChemCatChem 1:12.

## 161-BTP

Assessment of Methyl Red Degradation Activity by a Fusion Protein of *Rhodococcus opacus* 1CP azoreductase and *Candida boidinii* formate dehydrogenase

\*A. C. Ngo<sup>1</sup>, D. Tischler<sup>1</sup>, F. Schultes<sup>1</sup>, C. Mügge<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Microbial Biotechnology, Bochum, Germany

The presence of various azo dyes in the environment have been a long withstanding problem as it is toxic, mutagenic, and cause aesthetic pollution. Various approaches such as whole cell and enzymatic degradation are being investigated since these methods are more cost-effective and more environmental-friendly than known physical and chemical treatments. The enzyme azoreductase is being seen as a potential solution since it cleaves azo bond present in azo dyes. It uses NAD(P)H as a co-substrate to degrade dyes. Different microorganisms are known to produce this enzyme. Meanwhile, fusion proteins are composed of naturally or artificially fused protein subunits obtained from a single gene. It has a potential application in various industries as it provides practicality and optimizes enzymatic production. Therefore, this study aimed to check for the potential possibility of fusing azoreductase and formate dehydrogenase, wherein the latter allows NAD(P)H regeneration for the azoreductase to use for subsequent catalysis. The formate dehydrogenase and azoreductase genes were cloned to yield a single open reading frame into pET16bP vector including a His-tag as a linker. The construct was successfully transformed to *Escherichia coli* BL21 (DE3) cells and the fused gene was induced by 0.1 mM IPTG. The enzyme was assessed for its capability to degrade Methyl Red. This research can provide insights for a more efficient and optimized multienzyme system that has potential industrial purposes.

## 162-BTP

Heterologous production of the cholinomimetic drug physostigmine in *Myxococcus xanthus*

L. Winand<sup>1</sup>, \*M. Nett<sup>1</sup>

<sup>1</sup>TU Dortmund University, Department of Biochemical and Chemical Engineering, Dortmund, Germany

Myxobacteria are proficient producers of bioactive secondary metabolites with significant biotechnological potential. Unlike other similarly gifted microbes, however, the genetic engineering of myxobacteria is still at its infancy. Even though the chromosomal inactivation and integration of biosynthetic genes are in principle possible, the available methods are quite laborious and time-consuming.

To facilitate the engineering of these microorganisms, we recently developed a novel plasmid-based expression system for the model myxobacterium *Myxococcus xanthus*. In a pilot study, this system was used to introduce a heterologous biosynthesis gene into *M. xanthus*, which led to the biotransformation of the naturally occurring siderophore myxochelin B.<sup>1,2</sup> To test the boundaries of plasmid-based gene expression, we then attempted the reconstitution of the physostigmine pathway, which was originally described from *Streptomyces griseofuscus* and comprises eight open reading frames (ORFs).<sup>3</sup> A polycistronic plasmid was constructed and introduced into *M. xanthus*. Subsequently, physostigmine as well as its biosynthetic intermediates were



detected in culture extracts of the recombinant strain. Our results prove the feasibility of plasmid-based multigene expression in myxobacteria.

[1] J. Korp, L. Winand, A. Sester, M. Nett, *Appl. Environ. Microbiol.* **2018**, 84, e01789-18

[2] A. Sester, L. Winand, S. Pace, W. Hiller, O. Werz, M. Nett, *J. Nat. Prod.* **2019**, 82, 2544-2549

[3] J. Liu, T. Ng, Z. Rui, O. Ad, W. Zhang, *Angew. Chem. Int. Ed.* **2014**, 53, 136-139

## 163-BTP

### Photoinduced monooxygenation involving NADPH-FAD sequential electron transfer

\*S. Ernst<sup>1</sup>, S. Rovida<sup>2</sup>, A. Mattevi<sup>2</sup>, S. Fetzner<sup>1</sup>, S. L. Drees<sup>1</sup>

<sup>1</sup>Westfälische Wilhelms-Universität Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany

<sup>2</sup>University of Pavia, Biology and Biotechnology, Pavia, Italy

#### Introduction

Photocatalysis provides a multitude of perspectives for implementation in biocatalysis, however, the number of applicable light-responsive enzymes is still limited. Engineering of photoenzymes is often based on flavoproteins, whose natural cofactor absorbs blue light and can undergo photoreduction with various organic electron donors. A major obstacle for flavoprotein photoenzyme research is that the protein thoroughly shields the flavin cofactor, thereby suppressing photoreactivity. We studied the flavoprotein monooxygenase PqsL from *Pseudomonas aeruginosa*, which catalyzes the *N*-hydroxylation of an aromatic amine. The enzyme shows no intrinsic activity with NAD(P)H as cosubstrate, but catalytic activity fueled by NAD(P)H can be triggered by blue light illumination.

#### Objectives

We aimed to unravel the mechanism of light-induced catalysis of PqsL in order to provide a basis for future engineering of photoenzymes.

#### Methods and Results

Spectroscopic, chromatographic and oxymetric techniques for monitoring PqsL photoreduction and -activity were developed. By illuminating PqsL with blue light in presence of various electron donors, we found that PqsL prefers NAD(P)H and shows typical Michaelis-Menten kinetics. EDTA, a common electron source for flavin photoreduction is not accepted. Rapid UV/Vis and fluorescence spectroscopy of the photoreduction revealed the formation of a transient flavin semiquinone, which indicates a sequential electron transfer from NAD(P)H to the protein-bound FAD. This observation is further supported by kinetic isotope effects. X-ray crystallographic studies suggested that a single proline impedes the classical hydride transfer mechanism between NAD(P)H to FAD.

#### Conclusion

Our data provide evidence for an unprecedented photoreduction of protein-bound FAD by a radical mechanism. We propose that simultaneous binding of FAD and NAD(P)H, a photoreceptive flavin and the impairment of

the canonical hydride transfer from NAD(P)H to FAD are prerequisites for flavoprotein-mediated photocatalysis.

## 164-BTP

### Elucidating Biological Networks of *Vibrio campbellii* RT-1 and *Vibrio harveyi* RT-6 strains Conferring Pan-genome and Comparative genomic analysis

\*T. RAJENDRAN<sup>1</sup>, I. Dhinakaran<sup>1</sup>, J. C. H<sup>2</sup>, P. Lingesan<sup>2</sup>, S. Bhoopathy<sup>1</sup>, K. Chandrasekaran<sup>1</sup>, S. A. Louis<sup>1</sup>

<sup>1</sup>Sathyabama Institute of Science and Technology, Centre for Ocean Research, Chennai, India

<sup>2</sup>Sathyabama Institute of Science and Technology, Department of Bioinformatics, Chennai, India

*Vibrio* species are abundant in marine coastal waters, estuaries and marine sediments. *Vibrio campbellii* and *Vibrio harveyi* are foremost colonizers of vibriosis infection in aquaculture farms causing high mortality and economic losses. To acquire a greater understanding of these closely related species, in terms of phylogenetic analyses, evolutionary relationships, and functional features, we performed the pan-genome and comparative genome analysis of *Vibrio campbellii* RT-1 (Vc RT-1) and *Vibrio harveyi* RT-6 (Vh RT-6) strains using various bioinformatics perspectives. A next-generation sequencing (NGS) strategy was executed using Illumina HiSeq 2500 platform followed by core- and pan-genome analysis. *De novo* assembled Vc RT-1 strain were aligned and compared the gene level with the Vh RT-6 genome sequences, which was isolated from an infected shrimp in aquaculture farms located in the Tamil Nadu region of India. Concurrently, common and unique genes have been identified between the two strains based on single-copy genes. Furthermore, Protein-protein interaction (PPI) network biology has emerged as a classic and powerful framework to visualize and analyze large data clusters in novel ways in the identification of hub proteins with unprecedented plasticity. Network-associated approaches have revealed molecular and biological pathways that may influence the vibriosis infection, suggesting novel mechanisms that contribute to pathogenicity that may form the basis of new therapeutic and diagnostic approaches.

**Keywords:** Aquaculture; Vibriosis; Next-generation sequencing; Pan-genome; Network analysis.

## 165-BTP

Tailoring *Corynebacterium glutamicum* for the efficient production of malonyl-CoA-dependent small molecules

\*J. Marienhagen<sup>1</sup>, N. Kallscheuer<sup>1</sup>, L. Milke<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany

*Corynebacterium glutamicum* is an important organism in industrial biotechnology for the microbial production of bulk chemicals, in particular amino acids. Functional integration of plant-derived biosynthetic pathways also allows for the microbial synthesis of various plant polyphenols such as flavonoids or stilbenes either from supplemented phenylpropanoid precursor molecules or directly from glucose.

However, similar to other microorganisms engineered for plant polyphenol synthesis, insufficient malonyl-CoA supply is also limiting polyphenol production with *C. glutamicum*. To date, the antibiotic cerulenin inhibiting fatty acid synthesis is added during microbial cultivations to improve malonyl-CoA availability for product formation at lab-scale. Unfortunately,

supplementation of cerulenin is very costly, which prohibits large-scale microbial polyphenol production.

We extensively engineered the central carbon metabolism of *C. glutamicum* with a focus on the TCA-cycle and the fatty acid metabolism to increase malonyl-CoA availability for polyphenol synthesis. In the context of this work, rational metabolic engineering strategies and FACS-based high-throughput screenings using transcriptional biosensors were successfully combined, yielding strain variants accumulating high polyphenol concentrations without supplementation of cerulenin.

We believe that, availability of these new *C. glutamicum* platform strains opens the door towards microbial production of plant polyphenols as well as other high-value aromatic compounds from cheap carbon sources at larger scale.

#### References:

Kallscheuer N., Vogt M., Stenzel A., Gätgens J., Bott M., Marienhagen J. (2016). *Metab. Eng.* 38: 47-55.

Milke L., Aschenbrenner J., Marienhagen J. and Kallscheuer N. (2018). *Appl. Microbiol. Biotechnol.*, 102: 1575–1585.

Kallscheuer N., Kage H., Milke L., Nett M., Marienhagen J. (2019). *Appl. Microbiol. Biotechnol.* (epub ahead of print)

#### 166-BTP

##### Design of O<sub>2</sub>-tolerant hydrogenase with ferredoxin binding site for light-driven H<sub>2</sub>-production

\*E. Lettau<sup>1</sup>, B. Bühler<sup>2</sup>, L. Lauterbach<sup>1</sup>

<sup>1</sup>Technische Universität Berlin, Institut für Chemie, MVL, PC14, Berlin, Germany

<sup>2</sup>Helmholtz Center for Environmental Research, Department Solar Materials, Leipzig, Germany

**Introduction:** Hydrogenases convert reversibly molecular hydrogen into protons and electrons. Coupling hydrogenases with the photosystem would make light-driven hydrogen production possible. [1]. To link both systems, an electron mediator with a suitable redox potential for H<sup>+</sup>-reduction ( $E_0' = -413$  mV) is required. Ferredoxin (Fd,  $E_0' \approx -430$  mV) interacts with the O<sub>2</sub>-sensitive NAD(P)<sup>+</sup>-reducing hydrogenase from *Synechocystis* sp. PCC 6803 (SynSH) [2]. However, the exact Fd binding site of SynSH is unknown. Additionally, an O<sub>2</sub>-tolerant hydrogenase is required, because O<sub>2</sub> is produced during photosynthesis. Such an O<sub>2</sub>-tolerant hydrogenase is the NAD<sup>+</sup>-reducing hydrogenase from *Ralstonia eutropha* H16 (ReSH) [3].

**Objectives:** We aim to (1) determine the Fd binding site of SynSH and (2) combine both key features of SynSH and ReSH hydrogenases, namely Fd binding and O<sub>2</sub> tolerance.

**Methods:** Amino acid exchanges and deletion of subunits of potential Fd binding sites were performed. Chimeric ReSH derivatives were constructed, which contain subunits from SynSH related to Fd binding.

**Results:** We produced SynSH derivatives and a set of chimeric SHs in *R. eutropha* and purified them by using affinity chromatography. The chimeric SHs showed H<sub>2</sub>-dependent NAD<sup>+</sup>-reducing activity, which demonstrates intact electron transfer within the chimeric enzymes and thus the feasibility of our approach. Preliminary results of O<sub>2</sub>

tolerance studies and Fd to hydrogenase interaction will be presented.

**Conclusion:** Our study represents a synthetic system for coupling photosystem with hydrogenases for light driven H<sub>2</sub> production. Thus, our approach holds considerable potential for application of sustainable biofuel production in order to prepare humanity for the post-fossil fuel age.

#### References:

[1] O. Lenz, L. Lauterbach, S. Frielingsdorf, and B. Friedrich, *Biohydrogen*, (2015) 61–96.

[2] K. Gutekunst, X. Chen, K. Schreiber, U. Kaspar, S. Makam, and J. Appel, *J. Biol. Chem.* 289:4, (2014) 1930–1937.

[3] L. Lauterbach and O. Lenz, *J. Am. Chem. Soc.* 135:47, (2013) 17897–17905.

#### 167-BTP

PEGylation increases stability and pharmacokinetic properties of arginine deiminase of *Streptococcus pyogenes*

N. Irmscher<sup>1,2</sup>, K. Volkmar<sup>1</sup>, C. Maletzki<sup>2</sup>, B. Kreikemeyer<sup>1</sup>, R. Schwarz<sup>3</sup>, \*T. Fiedler<sup>1</sup>

<sup>1</sup>Universitätsmedizin Rostock, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Rostock, Germany

<sup>2</sup>Universitätsmedizin Rostock, Klinik III - Hämatologie, Onkologie, Palliativmedizin, Rostock, Germany

<sup>3</sup>Universitätsmedizin Rostock, Institut für Pharmakologie und Toxikologie, Rostock, Germany

#### Question

Arginine auxotrophy constitutes a metabolic defect that renders tumor cells vulnerable towards arginine-depleting substances, such as arginine deiminase (ADI) from *Streptococcus pyogenes*. Previously, we confirmed ADI-susceptibility on patient-derived glioblastoma multiforme (GBM) models *in vitro* and *in vivo*. Functionally, effects were attributable to induction of autophagy, senescence and necrosis. For application in patients, immunogenicity of the enzyme needs to be reduced and pharmacokinetic properties improved. For that purpose we conjugated the *S. pyogenes* derived ADI with polyethylene glycol moieties and investigated the impact of the PEGylation on kinetic properties, stability and *in vitro* antitumoral effects on GBM cells.

#### Methods

ADI from *S. pyogenes* M49 strain 591 was heterologously produced in *E. coli*, purified via StrepTactin affinity chromatography and PEGylated with mPEG20K-succinimidyl carboxymethyl ester. ADI-PEG20 was separated from native ADI via anion-exchange chromatography using a HiTrap Q XL column. V<sub>max</sub>, K<sub>m</sub> for arginine and stability were tested with enzymatic assays. Patient-derived GBM cells were treated for 2 x 72 h with 35 mU of ADI and ADI-PEG20, respectively. Cell mass was determined photometrically after crystal violet staining.

#### Results

With the chosen method, the *S. pyogenes* ADI is efficiently but not homogeneously PEGylated, resulting in ADI species

conjugated to one up to multiple PEG residues. PEGylated ADI can be separated from the unmodified enzyme by anion-exchange chromatography. PEGylation reduces the specific activity of the enzyme to about 50 % as compared to the unmodified enzyme. PEGylation however does not affect the kinetic properties of the enzyme but stabilizes the enzyme in long term storage, decreases proteolytic degradation and increases its *in vitro* antitumoral activity towards GBM cells.

## Conclusions

ADI from *S. pyogenes* is a potent proliferation inhibitor of arginine auxotrophic tumor cells. ADI stability and therefore its usability in animal models and patients is increased by conjugating the enzyme with PEG20.

## 168-BTP

Microfluidic platform designed for cultivation and integrated characterization of biofilms under various environments

\*E. Klein<sup>1</sup>, J. Weiler<sup>1</sup>, M. Edel<sup>1</sup>, H. Horn<sup>2</sup>, C. M. Niemeyer<sup>3</sup>, D. Rehn<sup>1</sup>, J. Gescher<sup>1,3</sup>

<sup>1</sup>Karlsruhe Institut of Technology (KIT), Institute for Applied Biology (IAB), Department of Applied Biology, Karlsruhe, Germany

<sup>2</sup>Karlsruhe Institute of Technology (KIT), Engler-Bunte-Institut, Wasserchemie und Wassertechnologie, Karlsruhe, Germany

<sup>3</sup>Karlsruhe Institute of Technology (KIT), Institute for Biological Interfaces (IBG-1), Eggenstein-Leopoldshafen, Germany

**Introduction:** Microbial cultivation with the aid of microfluidic flow chambers has a great potential to form biofilms on an easy to handle laboratory scale. Our microfluidic cultivation platform with a modular design offers versatility and precision in terms of simulating different environments to study multiple growth conditions. Thus, a long-term cultivation of biofilms can easily be obtained with the possibility of integrated on-line analysis.

**Objectives:** Our goal was to present two unique biofilm systems using the platform.

**Methods:** The channel geometry of the PDMS-based microfluidic chips can be chosen as required and combined modularly. Anoxic cultivation is possible as well as the use of gas as feeding source. The biofilms can be analyzed with different integrated analytical methods such as fluorescence *in situ* hybridization, optical coherence tomography and ion analysis.

**Results:** The integration of a switch system enabled the cultivation of biofilms under cyclic change of two media and gases according to the aerobic granular sludge (AGS) principle. During cultivation, samples were taken directly from the microfluidic cultivation channels with a robotic sampling device. Analysis of the cultured biofilms included ion analytical methods and spatiotemporal amplicon sequencing, as well as imaging at the micro- and mesoscale.

In a second approach, we show that electroactive microorganisms such as *Shewanella oneidensis* can be cultivated by incorporating electrodes into the cultivation channel. The extended respiratory chain of these microbes enables extracellular electron transfer from the cytoplasm through the cell membranes to an insoluble electron acceptor, such as an anode. Different deletion strains were cultivated and could be examined and compared with each other by current measurements and confocal laser scanning microscopy.

**Conclusion:** In summary, biofilm growth under both oscillating oxic/anoxic conditions as well as solely anoxic conditions using an anode as electron sink can be studied using our microfluidic flow technology. Examples of the versatility of the system as well as future outlooks on further biofilm growth conditions will be presented.

## 169-BTP

Immobilisation of Microbial Siderophores using Solid Phase Extraction Columns

\*L. Malik<sup>1</sup>, M. Hofmann<sup>1</sup>, D. Tischler<sup>1,2</sup>, M. Schlömann<sup>1</sup>, T. Heine<sup>1</sup>

<sup>1</sup>TU Bergakademie Freiberg, Ecological Center, Freiberg, Germany

<sup>2</sup>Ruhr University Bochum, Microbial Biotechnology, Bochum, Germany

**Introduction.** Siderophores are natural chelating compounds having a high affinity to iron(III) and further metal ions. According to their complexing properties, siderophores are applied in many fields including medicine, agriculture, bioremediation, ecology, and biosensors. Attachment to solid phases can simplify the utilisation of these metalophores and enables continuous processes.

**Objectives.** The siderophores produced by *Rhodococcus erythropolis* B7g, *Variovorax paradoxus* EPS and *Pseudomonas chlororaphis* DSM 50083 were immobilised using three different types of solid phase extraction columns.

**Materials and Methods.** Siderophore containing culture supernatants were applied to NH<sub>2</sub>, silica and C<sub>18</sub> columns and eluted by increasing the methanol concentration. To determine the optimal binding conditions the pH value and salt content were varied. The amount of unbound siderophore in the collected fractions was quantified via chrome azurol S (CAS) -assay. The columns with the best immobilisation results were loaded with a defined amount of Fe(III) to investigate the metal binding ability of the immobilised siderophores. ICP-MS measurement was used to analyse the metal content of the flow through.

**Results.** The best immobilisation of *R. erythropolis* B7g siderophore was achieved by using silica and C<sub>18</sub> columns at pH 2. In the case of *V. paradoxus* EPS, only C<sub>18</sub> columns were able to bind 100 % of the loaded siderophore. The immobilisation of *P. chlororaphis* DSM 50083 siderophore on C<sub>18</sub> columns enabled the best results for this strain and was almost pH-value independent. All three siderophores were still active in bound state.

**Conclusion.** In general, acidic supernatants applied to C<sub>18</sub> columns showed the best immobilisation results, while NH<sub>2</sub> solid phases did not bind any of the investigated siderophores in a sufficient way. The salt content had no major impact on the binding process.

## 170-BTP

A highly active UDP-glucose pyrophosphorylase from *Thermocrispum agreste* DSM 44070 - TaGalU

\*A. Kumpf<sup>1</sup>, D. Kowalczykiewicz<sup>2</sup>, K. Szymanska<sup>3</sup>, M. Mehnert<sup>4</sup>, I. Bento<sup>5</sup>, A. Pollender<sup>4</sup>, A. Łochowicz<sup>3</sup>, A. Jarzębski<sup>3</sup>, D. Tischler<sup>1,4</sup>

<sup>1</sup>Ruhr-Universität Bochum, Mikrobielle Biotechnologie, Bochum, Germany

<sup>2</sup>Silesian University of Technology, Department of Organic Chemistry, Gliwice, Poland

<sup>3</sup>Silesian University of Technology, Department of Chemical and Process Engineering, Gliwice, Poland

<sup>4</sup>TU Bergakademie Freiberg, Institut für Biowissenschaften, Freiberg, Germany

<sup>5</sup>EMBL Hamburg, Hamburg, Germany

Activated sugar moieties are of importance for various (bio)synthetic routes as for example sugar and starch metabolism or biosynthesis of antibiotics. One of the most important activated sugars is UDP-glucose, which can be converted by UDP-glucose pyrophosphorylase (GalU; EC 2.7.7.9) out of UTP and glucose 1-phosphate.

For the production, either free GalU can be used or microbial cells. However, often the cell metabolism does not allow overproducing activated sugars or secretion and thus downstream processing is complicated. Here enzyme catalysis comes into focus and in order to stabilize the enzyme, as well as to make the overall process economical feasible, enzyme immobilization is one of the major strategies to provide a proper biocatalyst.

Besides a robust immobilization method, a highly active and stable GalU enzyme is needed to efficiently produce UDP-glucose, which can then be employed to produce other compounds such as trehalose or UDP-glucuronate.

By following a genome mining approach the *galU* gene of the thermostable soil actinobacterium *Thermocristum agreste* DSM 44070 (*TaGalU*) was identified. It was codon optimized and expressed in the recombinant host *E. coli* BL21(DE3) pLysS, the protein was biochemically characterized, as well as immobilized on micro cellular foams (MCF) *via* either epoxy or amino group functionalization. Only the latter led to an active and useful biocatalyst.

The soluble and highly active *TaGalU* revealed a  $V_{max}$  of 1698 U  $mg^{-1}$  (UTP) and a  $K_m$  of 0.15 mM (UTP). The optimum reaction temperature was determined to be 50 °C. *TaGalU* was stable at this temperature for up to 30 min with a maximum loss of activity of 65 %. Interestingly, immobilized *TaGalU* was stable at 50 °C for at least 120 min without a significant loss of activity. Further, the immobilized *TaGalU* had a specific activity of 1445 U  $mg^{-1}$ .

In conclusion, the genome mining approach was successful, as the highest GalU activity was found compared to literature. An increased stability and retained activity with a stable conversion rate of 90 % after five subsequent reaction cycles made the immobilized *TaGalU* an interesting biocatalyst for chemical synthesis of UDP-glucose.

## 171-BTP

Generation of multifunctional magnetic nanoparticles by magnetosome display of peptides and reporter enzymes

\*C. Lanzloth<sup>1</sup>, F. Mickoleit<sup>1</sup>, D. Schüler<sup>1</sup>

<sup>1</sup>Universität Bayreuth, Mikrobiologie, Bayreuth, Germany

Magnetotactic bacteria like the alphaproteobacterium *Magnetospirillum gryphiswaldense* biosynthesize magnetosomes for their navigation along magnetic field lines. Magnetosomes consist of a monocrystalline magnetite core, which is surrounded by a biological membrane. The latter harbours a set of specific proteins with important functions in magnetosome biosynthesis [1]. Due to subtle control on each of biomineralization, particles with unprecedented properties like uniform sizes and shapes, as well as high crystallinity and strong magnetization are formed. In addition, the composition of both the magnetite core as well as the magnetosome membrane can be manipulated by genetic engineering. Foreign proteins are expressed as translational fusions to highly abundant magnetosome membrane (Mam) proteins, which serve as membrane anchors. Because of these characteristics, magnetosomes have already been envisioned for many

applications in the biomedical and biotechnological field. The latter would benefit from multimodal particles that in addition to their magnetic properties simultaneously display functional moieties on the surface, such as enzymes, receptors or antibodies.

For a selective and controllable functionalization of the magnetosome surface, an optimized expression system was developed [2], utilizing highly abundant Mam proteins as anchors for the surface display of foreign peptides and enzymes. The versatility of this approach was demonstrated by the simultaneous expression of different gene fusions, thereby creating magnetic "model" nanoparticles with several fully genetically encoded functionalities, i.e. different enzymatic activities, fluorescence and coupling groups (nanobodies) that allow binding of any complementary-tagged group and thus, the generation of new composite materials.

In summary, this illustrates the versatile features of engineered bacterial magnetosomes, with enhanced potential for numerous biomedical or biotechnological applications.

[1] Uebe R., Schüler D. (2016) *Nat. Rev. Microbiol.*, 14(10), 621.

[2] Borg S., Hofmann J., Pollithy A., Lang C., Schüler D. (2014) *Appl. Environ. Microbiol.* 80(8), 2609.

## 172-BTP

Metabolic engineering of *Corynebacterium glutamicum* for high-level ectoine production: design, combinatorial assembly, and implementation of a transcriptionally balanced heterologous ectoine pathway

\*L. Jungmann<sup>1</sup>, G. Gießelmann<sup>1</sup>, D. Dietrich<sup>1</sup>, M. Kohlstedt<sup>1</sup>, E. J. Jeon<sup>2</sup>, S. S. Yim<sup>2</sup>, F. Sommer<sup>3</sup>, D. Zimmer<sup>3</sup>, T. Mühlhaus<sup>3</sup>, M. Schroda<sup>3</sup>, K. J. Jeong<sup>2</sup>, J. Becker<sup>1</sup>, C. Wittmann<sup>1</sup>

<sup>1</sup>Saarland University, Institute for Systems Biotechnology, Saarbrücken, Germany

<sup>2</sup>KAIST, Chemical and Biomolecular Engineering, Daejeon, South Korea

<sup>3</sup>TU Kaiserslautern, Molecular Biotechnology and Systems Biology, Kaiserslautern, Germany

**Introduction:** Microbes produce ectoine as compatible solute to cope with environmental stress like extreme osmolarity and temperature. Since many years, ectoine is a product of recognized commercial value for cosmetics and pharmaceuticals, due to its outstanding ability to protect macromolecules, cells, and tissues. Commercial ectoine manufacturing today is based on a complex high-salinity process using the extremophilic bacterium *Halomonas elongata*.

**Objectives:** The aim of this work was to establish a simple low-salt production process for ectoine using *Corynebacterium glutamicum*. For this purpose, transcriptional fine-tuning of the heterologous *ectABC* gene cluster from *Pseudomonas stutzeri* aimed to create a superior producer.

**Methods:** An ectoine pathway library was constructed, randomly combining each gene of the *ectABC* cluster with one out of 19 synthetic promoters and one out of three linker sequences, theoretically yielding 185000 unique combinations. The library was transformed into *C. glutamicum*, followed by screening of hundreds of mutants for production performance and genomic and proteomic analysis of most promising candidates.

**Results:** The analysed 400 clones ranged from very low to high ectoine production and included several candidates, which surpassed the control strain with a conventional

monocistronic *ectABC* expression design more than 5-fold. Enhanced ectoine production was supported by a strong expression of the *ectB* gene and specific balance in the expression levels of *ectA* and *ectB*. In a fed-batch process, the best strain *C. glutamicum* *ectABC*<sup>opt</sup> achieved a final ectoine titer of 65 g L<sup>-1</sup> within 55 hours [1]. **Conclusion:** Transcriptional balancing of the biosynthetic pathway was identified as a key to drive ectoine formation. The created strain *C. glutamicum* *ectABC*<sup>opt</sup> is a milestone towards industrial production of ectoine at low salt levels.

[1] Giesselmann et al., *Biotechnol. J.* 2019, 14(9), 1800417

### 173-BTP

A timed off-switch for dynamic control of gene expression in *Corynebacterium glutamicum*

\*D. Siebert<sup>1</sup>, J. Altenbuchner<sup>2</sup>, B. Blombach<sup>1</sup>

<sup>1</sup>Technische Universität München, Professur Mikrobielle Biotechnologie, Straubing, Germany

<sup>2</sup>Universität Stuttgart, Institut für Industrielle Genetik, Stuttgart, Germany

### Introduction

Dynamic control of gene expression mainly relies on inducible systems which require supplementation of (costly) inducer molecules such as IPTG. In contrast, synthetic regulatory circuits which allow the timed shutdown of gene expression are rarely available and therefore represent highly attractive tools for metabolic engineering. *Corynebacterium glutamicum* uses the cheap compound vanillic acid as carbon and energy source (Merkens et al., 2005). Expression of the *vanABK* operon which is required for vanillic acid utilization is activated by the transcriptional regulator VanR in the presence of vanillic acid (Heravi et al., 2014). Since vanillic acid is co-metabolized with e.g. glucose, this regulatory circuit represents an elegant system to turn-off gene expression when vanillic acid is depleted.

### Methods and Results

The pyruvate dehydrogenase complex (PDHC) is essential for growth of *C. glutamicum* on glucose and was previously shown to be an attractive target to improve pyruvate availability for the production of e.g. L-valine or isobutanol (Eikmanns and Blombach, 2014). To control expression of the PDHC in a vanillic acid-dependent manner, we replaced the native promoter region of the *aceE* gene, which encodes the E1p enzyme of the PDHC, by *vanR* and the altered promoter P<sub>vanABK</sub>. Growth experiments in minimal medium with glucose and varying amounts of vanillic acid (1 to 20 mM) revealed a clear concentration-dependent growth phenotype of the mutant strain; i.e. cell proliferation stopped when vanillic acid was metabolized. As expected, when growth ceased due to consumption of vanillic acid, the mutant strain utilized the residual glucose to produce significant amounts of pyruvate, L-alanine and L-valine.

### Conclusions

As shown in our study, the regulatory circuit based on *vanR* and the mutated promoter P<sub>vanABK</sub> represents a useful metabolic engineering tool to shutdown gene expression with vanillic acid as cheap and abundant effector molecule.

### References

Merkens H, et al. doi: 10.1007/s00284-005-4531-8.

Heravi KM, et al. doi: 10.1128/JB.02431-14.

Eikmanns BJ and Blombach B. doi: 10.1016/j.jbiotec.2013.12.019.

### 174-BTP

BioTrans: Biodiversity of Iranian soil bacteria with  $\omega$ -Transaminases for the production of chiral amines

N. Gord Noshari<sup>1</sup>, U. Engel<sup>2</sup>, \*J. Rudat<sup>2</sup>

<sup>1</sup>Alzahra University, Department of Biotechnology, Faculty of Biology Science, Tehran, Iran

<sup>2</sup>Karlsruhe Institute of Technology (KIT), BLT\_II: Technical Biology, Karlsruhe, Germany

Chiral amine building blocks are essential elements of approximately 40% of new chemical entities in recent years [1].  $\omega$ -Transaminases ( $\omega$ -TA) are promising enzymes for the synthesis of chiral amines, amino acids and amino alcohols from prochiral substrates [2].

Major challenges in the application of  $\omega$ -TA are a restricted substrate scope for large molecules as well as rather low enzyme stability under extreme reaction conditions associated with pH, temperature and organic solvents [3, 4]. Iranian landscapes contain highly diverse microbial storages due to considerable variety in geographical features and thus can be considered a rich source for special extremophile biocatalysts [5].

In this study, enrichment cultures from Iranian soils using (*rac*)- $\alpha$ -methylbenzylamine (MBA) as sole nitrogen source were screened for  $\omega$ -TA activity by a colorimetric assay. The most promising strains were identified by 16S rDNA sequencing and subjected to determine optimal conditions for  $\omega$ -TA activity.

A *Bacillus halotolerans* strain showed a highly versatile substrate spectrum toward a plethora of aromatic amino donors over a wide pH range, exhibiting excellent solvent tolerance [6]. In contrast to other  $\omega$ -TA that usually show their maximum activity at slightly alkaline pH, two strains with high similarity to *B. endophyticus* and *B. stercoris* showed highest  $\omega$ -TA activity under acidic conditions with 70% remaining activity at pH 3.

To the best of our knowledge, this is the first report on  $\omega$ -TA activity under acidic conditions.

[1] Aleku GA, France SP, Man H, Mangas-Sanchez J, Montgomery SL, Sharma M, Leipold F, Hussain S, Grogan G, Turner NJ (2017) *Nat Chem* 9: 961.

[2] Guo F, Berglund P (2016) *Green Chem* 19: 333

[3] Pavlidis IV, Weiß MS, Genz M, Spurr P, Hanlon SP, Wirz B, Ilding H, Bornscheuer, U (2016) *Nat Chem* 8: 1076

[4] Kelly SA, Magill DJ, Megaw J, Skvortsov T, Allers T, McGrath JW, Allen CC, Moody TS, Gilmore BF (2019) *Appl Microbiol Biotechnol* 103: 5727

[5] Ataee N, Fooladi J, Namaei MH, Rezadoost H, Mirzajani F (2017) *Biocatal Agric Biotechnol* 9: 127

[6] Gord Noshari N, Fooladi J, Syltatk C, Engel U, Heravi MM, Mehrjerdi MZ, Rudat J (2019) *Catalyst* 9: 874

---

## 175-BTP

### Systems metabolic engineering of *Corynebacterium*

#### *glutamicum* for the production of L-Lysine derived products

\*S. Pauli<sup>1</sup>, C. M. Rohles<sup>1</sup>, G. Gießelmann<sup>1</sup>, M. Kohlstedt<sup>1</sup>, J. Becker<sup>1</sup>, C. Wittmann<sup>1</sup>

<sup>1</sup>Saarland University, Institute for Systems Biotechnology, Saarbrücken, Germany

**Introduction.** The soil bacterium *Corynebacterium glutamicum* is a major workhorse in industrial biotechnology. Interesting products today include 5-aminovalerate and glutarate, which have recognized value as building blocks for bio-based plastics [1].

**Objectives.** *De novo* 5-aminovalerate production should be enabled using the engineered L-lysine producer *C. glutamicum* LYS-12 as chassis [2]. The conversion of L-lysine into the product of interest should be catalyzed by heterologously expressing L-lysine 2-monooxygenase (*davB*) and 5-aminovaleramide amidase (*davA*) from *Pseudomonas putida* together with the endogenous 5-aminovalerate transaminase (*gabT*) and glutarate semialdehyde dehydrogenase (*gabD*) [3].

**Materials & methods.** An interdisciplinary set of methods including genetic and metabolic engineering, enzyme analysis, process optimization, and analytics was used to create and characterize the strains.

**Results.** A microbial 5-aminovalerate cell factory could be established successfully. *C. glutamicum* AVA-3 accumulated 5-aminovalerate at a yield of 275 mmol mol<sup>-1</sup>, after deletion of the native L-lysine exporter and the endogenous 5-aminovalerate transaminase. In a fed-batch fermentation the strain produced 28 g L<sup>-1</sup> 5-aminovalerate with a maximal space-time yield of 0.9 g L<sup>-1</sup> h<sup>-1</sup>. The high performance was supported by a well-balanced activity of DavB and DavA. Enzyme studies identified 5-aminovalerate as inhibitor of DavB activity, leaving space for further improvement.

**Conclusion.** The newly derived strain *C. glutamicum* AVA-3 enabled *de novo* production of 5-aminovalerate. In future studies, the elimination of the undesired feedback inhibition of L-lysine 2-monooxygenase could contribute to further strain optimization.

[1] Rohles, CM, *et al.* (2018). *Green Chem.* **20**:4662.

[2] Becker, J, *et al.* (2011). *Metab. Eng.* **13**:159.

[3] Rohles, CM, *et al.* (2016). *Microb. Cell Fact.* **15**:154.

---

## 176-BTP

### Development of an enzyme cascade process for biocatalytic production of chiral beta amino acids

U. Engel<sup>1</sup>, \*J. Rudat<sup>1</sup>

<sup>1</sup>Karlsruher Institut für Technologie (KIT), IBLT II: Technische Biologie, Karlsruhe, Germany

### Development of an enzyme cascade process for biocatalytic production of chiral beta amino acids

Jens Rudat, Ulrike Engel

Chiral  $\beta$ -amino acids are valuable building blocks for pharmaceuticals and fine chemicals (Rudat, Brucher, &

Syldatk, 2012). Within this project chiral  $\beta$ -amino acids are to be produced applying a modified hydantoinase process using racemic dihydropyrimidines as educts. The process is to be based on two enzymes. A dihydropyrimidinase will be used for hydrolytic cleavage of the dihydropyrimidine ring followed by the reaction of a linear amidase able to decarbamoylate *N*-carbamoyl  $\beta$ -amino acids. Both enzymes are to be immobilized and applied in a microfluidic system.

It was already demonstrated in previous work that hydantoinases/dihydropyrimidinases can hydrolyze racemic 6-substituted dihydropyrimidines to the corresponding *N*-carbamoylated  $\beta$ -amino acids (Engel, Syldatk *et al.* 2012). For several hydantoinases the determination of kinetic parameters for the model substrate phenyldihydrouracil, the evaluation of optimal reaction conditions and immobilization experiments are ongoing.

Within a screening novel decarbamoylating enzymes were discovered, recombinantly expressed and characterized. It was shown that these enzymes hydrolyze several different novel *N*-carbamoyl- $\beta$ -amino acids as well as *N*-carbamoyl- $\beta$ -homo-amino acids to their corresponding  $\beta$ -amino acids. A comparison of these enzymes gave novel insight into their mechanism and biological function.

In upcoming work the enzyme cascade consisting of hydantoinase and decarbamoylating enzyme is to be assembled, characterized and optimized in order to produce chiral  $\beta$ -amino acids.

Rudat, J., Brucher, B.R. & Syldatk, C., *AMB Express*, **2**(1), (2012) p.11.

Engel, U., Syldatk, C. and Rudat, J., *Appl. Microbiol. Biotechnol.*, **94**(5), (2012), 1221-1231.

---

## 177-BTP

### Enantioselective epoxidation of poorly water-soluble substrates by flavin-dependent monooxygenases in the presence of organic solvents

\*D. Eggerichs<sup>1</sup>, J. Mayweg<sup>1</sup>, D. Tischler<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Mikrobielle Biotechnologie, Bochum, Germany

Styrene monooxygenases catalyze the introduction of a single oxygen atom into an organic molecule using atomic oxygen as cosubstrate. After each reaction cycle, the flavin cofactor is reduced by a reductase on the expense of NADH. Because water is the only side product of the reaction, the process is overall environmentally friendly. In contrast, the enantioselective functionalization of organic molecules is still a large hurdle for classical chemical processes which can only be overcome by the price of toxic transition metal-based catalysts and a huge waste production. Thus, in terms of sustainability and the transition to green chemistry, enzymes gain more and more attention for the use in chemical industry.

But so far, many substrates are only poorly soluble in aqueous solutions and by this not accessible for enzymes. So, the usage in industry requires often the addition of organic cosolvents. Due to this issue, several strategies were developed to improve either the stability of the enzyme (e. g. directed evolution, immobilization) or to optimize the reaction conditions for the enzyme (e. g. two phase system).

Here, the reaction conditions of the solvent-tolerant indole monooxygenase A from *Gemmobacter nectarophilus* (GnlndA) were optimized. For this, the enzyme was obtained in pure form by a His-tag purification and used in two-hour biotransformations with the artificial NADH mimic 1-benzyl-1,4-dihydronicotinamide (BNAH) as electron donor.

HPLC analysis revealed a stable product formation in the presence of up to 20 vol% cosolvent. Furthermore, additional solvents increased the product amount in a concentration and hydrophobicity (log PO/W) dependent manner. An applied concentration of 10 vol% methanol increased the product amount about +56% while the same concentration of 1-propanol caused a decrease of more than -90%. At the same time, the enzymes enantiomeric excess of GnlndA was determined by chiral GC-FID and is not influenced by the applied solvent.

In conclusion, our results indicate a correlation between cosolvent-mediated substrate accessibility and enzymatic activity. These results make GnlndA a promising candidate for further applications.

---

### 178-BTP

#### Evidence for a unique PDH/ODH supercomplex in *Corynebacterium glutamicum*

\*L. Sundermeyer<sup>1</sup>, M. Baumgart<sup>1</sup>, M. Bott<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences: Biotechnology (IBG-1), Jülich, Germany

**Introduction:** The corynebacterial 2-oxoglutarate dehydrogenase (ODH) differs from most other bacterial ODHs. The typical ODH complex consists of three different subunits, the 2-oxoglutarate decarboxylase (E1 $\alpha$ ), a dihydrolipoyl transsuccinylase (E2 $\alpha$ ), and the dihydrolipoyl dehydrogenase (E3), which is shared between ODH and the pyruvate dehydrogenase complex (PDH). In *C. glutamicum*, E1 $\alpha$ , called OdhA, is fused with the succinyltransferase domain of E2, while a separate E2 $\alpha$  subunit is missing. Since OdhA lacks the lipoyl-binding domains of a standard E2 $\alpha$ , an interaction of OdhA with the E2 subunit (AceF) of PDH is necessary for ODH activity [1,2].

**Objectives:** Since ODH and PDH both play an important role in central metabolism, an important aim of our research is to gain further insights into the structure of these dehydrogenase complexes and how AceF and LpdA are shared between ODH and PDH.

**Materials & methods:** The integration vector pK18mob was used to add tags for affinity purification to the genes *odhA*, *aceE* and *aceF* in the genome of *C. glutamicum*. These tagged versions of OdhA, AceE and AceF were used for affinity chromatography to analyse the protein composition of the ODH and PDH complexes. The enzymatic activities of the purified dehydrogenase complexes were analysed in NAD<sup>+</sup> dependent activity assays.

**Results:** The purification of Strep-tagged OdhA enabled the copurification of AceF and LpdA as well as the copurification of the PDH E1 subunit AceE. Furthermore, the protein eluate possessed PDH activity of 0.3 U/mg besides the expected ODH activity of 2.5 U/mg. Copurification experiments with AceE-Strep as well as the shared E2 subunit AceF also enabled the copurification of active PDH and ODH complexes.

**Conclusion:** The observation that it is possible to purify both dehydrogenase complexes in an active state with one of the

E1 subunits suggests that AceF and LpdA are not only part of both dehydrogenase complexes, but that the four proteins OdhA, AceE, AceF and LpdA form a metabolic supercomplex possessing ODH and PDH activity.

[1] Niebisch et al. (2006) J Biol Chem 281: 12300–12307

[2] Hoffelder et al. (2010) J Bacteriol 192: 5203–5211

---

### 179-BTP

#### Heterologous Production of the Polyphenol Pinosylvin with *Pseudomonas taiwanensis* VLB120

\*T. Schwanemann<sup>1</sup>, M. Otto<sup>1</sup>, N. Wierckx<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich GmbH, IBG-1, Microbial Catalysis, Jülich, Germany

The production of polyphenols from plants is mainly based on isolation processes from plants. These approaches are limited by laborious, wasteful purification procedures and low yield. The heterologous production of stilbenes in microbial hosts is frequently demonstrated in popular bacterial workhorses although these approaches are faced with multiple major drawbacks like product- or intermediate toxicities and poor precursor supply, requiring costly supplementation.

Pinosylvin, a stilbene from coniferous wood, has various health promoting effects including antimicrobial activity. The bacterium *Pseudomonas taiwanensis* VLB120 shows astonishing resistance against this polyphenol compared to *Escherichia coli*. A genome-reduced-chassis strain of *P. taiwanensis* with improved bioprocess features surpasses even the wildtype in resistance and is an established and reliable host for the efficient production of aromatic amino acids. The availability of aromatic amino acids or phenylpropanoids for the synthesis of stilbenes often limits their cost-effective heterologous microbial production. Thus, a phenylalanine overproducing strain of *P. taiwanensis* is elaborated to constitutively produce pinosylvin as a representative polyphenol without the requirement of antibiotics. This approach reaches comparable titers to T7-driven, plasmid-based published results. Metabolic engineering and process optimization is carried out to enhance precursor availability further and increase polyphenol production titers in this unique host.

---

### 180-BTP

The electric-gut microbiome: selecting electroactive microorganisms from mouse feces

\*L. Rago<sup>1</sup>, J. T. Heiker<sup>2</sup>, D. Popp<sup>1</sup>, F. Harnisch<sup>1</sup>

<sup>1</sup>UFZ - Helmholtz Zentrum für Umweltforschung, UMB, Leipzig, Germany

<sup>2</sup>Helmholtz Institute for Metabolic, Obesity and Vascular Research (HI-MAG), Helmholtz Zentrum München an der Universität Leipzig und dem Universitätsklinikum Leipzig AöR, Leipzig, Germany

#### Introduction

Electroactive microorganisms (EAM) are performing extracellular electron transfer (EET) representing a unique metabolic trait. Although the most studied EAM are belonging to *Geobacter* and *Shewanella* genera, many more species were recognized as EAM. It was also demonstrated that there is not a specific ecological niche for EAM<sup>1</sup>. Thus, more electroactive species presumably exist, and they can be found in complex ecological niches, e.g. the mammalian gut microbiome. Recently, we demonstrated in pure culture studies that *Clostridium cochlearium*, *Lactobacillus reuteri*

and *Staphylococcus xylosus* -being inherent to the mouse gut microbiome- are EAM<sup>2</sup>.

## Objectives

With the aim of the identification of new -so far unknown- EAM, a follow-up study for the electrochemically driven selection from mouse feces was performed.

## Materials & methods

Glucose-fed bioelectrochemical reactors (n=3) were inoculated with the feces of male mice (C57BL/6N) and operated for 4 fed-batch cycles (~one week for each), applying a potential of +0.2V vs. Ag/AgCl sat. KCl. Control reactors (n=3) were run in parallel without the applying any potential (open circuit condition). For characterizing the mode of EET, cyclic voltammetry (CV) was performed. The DNA of the inoculum, of the planktonic cells collected at the end of each cycle and of the anodes (after the 4<sup>th</sup> cycle) was extracted and analyzed with 16S rRNA gene amplicon sequencing (Illumina MiSeq).

## Results

A maximum current density of 122±23mA cm<sup>-2</sup> after 19h was achieved and related to glucose consumption. After the 4th cycle, the presence of an anodic biofilm was demonstrated by optical microscopy. CV analysis revealed contributions of biofilm and planktonic cells to EET. Genetic analysis is still ongoing.

## Conclusion

We have provided further evidence on the presence of EAM in the mammalian gut. The future study of their diversity, metabolic pathways and trophic interactions, will shed further light and may bring to the possibility of designing prebiotic drugs for improved diet.

<sup>1</sup>C. Koch, F. Harnisch; *ChemElectroChem* 3 (2016), 1282-1295; <sup>2</sup>L. Schwab, L. Rago, C. Koch, F. Harnisch; *Bioelectrochemistry* 130 (2019), art.107334

## 181-BTP

Metabolic network analysis of mannose-grown

### *Corynebacterium glutamicum*

\*P. Cao<sup>1</sup>, S. Schwechheimer<sup>1</sup>, J. Becker<sup>1</sup>, C. Wittmann<sup>1</sup>

<sup>1</sup>Institute of Systems Biotechnology, Saarland University, Saarbrücken, Germany

**Introduction:** The soil bacterium *C. glutamicum* is an industrial producer for a broad spectrum of added-value products [1]. Efforts in metabolic engineering of *C. glutamicum* over the past decades have greatly benefited from a detailed understanding of its cellular physiology, mainly using glucose and sucrose as raw materials [2] [3]. For a sustainable bio-economy, we need, however, more than glucose and sucrose. Spent sulfite liquor (SSL) is a potentially attractive waste stream of the forestry industry to be used by *C. glutamicum* for industrial bio-production. It is a crude mixture of different sugars and contains mannose as major ingredient.

**Objectives:** Naturally, *C. glutamicum*, grows very poor on mannose, hence, we need to overcome the bottleneck and understand the central metabolism with mannose as carbon source.

**Materials & methods:** Genetic engineering, <sup>13</sup>C metabolic flux analysis and in silico pathway modelling using elementary flux modes.

**Results:** Here, we have created a mutant, which exhibits over expression of *manA* encoding mannose-6-phosphate isomerase for enhanced interconversion of mannose-6-phosphate and fructose-6-phosphate [4]. The genomic modification restored mannose-based growth and the intracellular flux distributions of the newly created metabolism on mannose were determined.

**Conclusion:** The data provide interesting insights into the physiology of the microbe and promise valuable knowledge for industrial use of SSL based raw materials.

## Acknowledgement

This project has received funding from the Bio Based Industries Joint Undertaking (JU) under grant agreement No 790507. The JU receives support from the European Union's Horizon 2020 research and innovation program and the Bio Based Industries Consortium.

## References

- [1] Becker, J , et al (2018). *Metab Eng.* 50, 122-141.
- [2] Wittmann, C , et al (2004). *Appl Environ Microbiol.* 70, 2777-87.
- [3] Kiefer, P , et al (2004). *Appl Environ Microbiol.* 70, 229-39.
- [4] Sasaki, M , et al (2011). *Appl Microbiol Biotechnol.* 89, 1905-16.

## 182-BTP

Synthesis of the alternative sweetener 5-ketofructose from sucrose by fructose dehydrogenase and invertase producing *Glucanobacter* strains

\*J. Hoffmann<sup>1</sup>, U. Deppenmeier<sup>1</sup>

<sup>1</sup>Institute for Microbiology and Biotechnology , University of Bonn, Bonn, Germany

In the last decades, a rapid increase of first-world diseases such as insulin resistance, type 2 diabetes or obesity linked to sugar-added food and sweetened beverage consumption was observed<sup>1</sup>. Hence, there is a crucial demand for low-calorie, sugar-free foods containing nonnutritive sweeteners. A promising alternative to artificial sweeteners is the microbially produced fructose derivative 5-ketofructose (5-KF), which possesses a very natural sweetness without any aftertaste. The compound was already successfully produced from fructose by overproduction of the key enzyme fructose dehydrogenase (Fdh) in *G. oxydans*<sup>2</sup>. For a renewable and cost-efficient production, we developed a method for the synthesis of 5-KF from sucrose, based on a biotransformation process with two different *G. oxydans* strains<sup>3</sup>. However, it is complicated to standardize parameters for a stable industrial production by using two strains due to deviating growth behaviors. In order to generate a single-strain system for the synthesis of 5-KF, the *fdh* genes were integrated into the chromosome of *G. oxydans* (*G. oxydans* Δ*mgdh::fdh*). The mutant showed a Fdh activity of 404 ± 10 mU/mg cell protein, which corresponded to 56% of the activity of *G. oxydans* *fdh* used in the previous system<sup>3</sup>. Since *G. oxydans* did not contain a



sucrose-hydrolyzing enzyme, several *Gluconobacter* strains were screened. We detected a novel sucrose-hydrolyzing enzyme in *G. japonicus* LMG1417, showing the highest specific activity compared to all mesophilic invertases described so far ( $V_{\max} = 2295 \pm 243$  U/mg protein). The corresponding gene was expressed in *G. oxydans*  $\Delta mgdh::fdh$ , resulting in an efficient production of 5-KF from sucrose. Overall  $84 \pm 2\%$  of the available fructose units of sucrose were converted to 5-KF.

<sup>1</sup>Aragno & Mastrocola, 2017. *Nutrients* 9:385

<sup>2</sup>Herweg et al. 2018. *Bioresource Technol.* 259:164-172

<sup>3</sup>Siemen et al. 2018. *Appl Microbiol Biotechnol.* 102:1699-1710

### 183-BTP

***Dictyostelium discoideum*** as a source of natural products and expression host for polyketide synthases from amoebozoa

\*C. Reimer<sup>1,2</sup>, J. E. Kufs<sup>2,3</sup>, J. Rautschek<sup>3</sup>, V. Valiante<sup>3</sup>, F. Hillmann<sup>1</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute (HKI), Evolution of Microbial Interactions, Jena, Germany

<sup>2</sup>Friedrich Schiller University, Jena, Germany

<sup>3</sup>Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Biobricks of Microbial Natural Product Syntheses, Jena, Germany

Polyketides are bioactive natural products mainly derived from fungi, bacteria or plants. Genome sequencing among the amoebozoa has revealed an unprecedented diversity of polyketide synthase (PKS) genes with a hidden potential for natural products and novel biosynthetic pathways. The model amoeba *Dictyostelium discoideum* encodes up to 45 PKS genes but their enzymatic products have remained largely unknown, as most of them are transcriptionally silent during vegetative growth under laboratory conditions. In order to analyze the PKS genes and elucidate novel polyketide-derived products, several PKS overexpression mutants of *Dictyostelium* were generated and analyzed for the presence of new secondary metabolites by HPLC and High-Resolution Mass Spectrometry. The gene encoding PKS37 was selected as a proof of principle since *in vitro* studies already showed Phlorocaprophenone (PCP) as the polyketide product. The metabolic analysis of the PKS37 overexpression strain revealed high levels of PCP which was further validated by NMR-guided structure elucidation. In addition, the overexpression of two uncharacterized PKS genes in *D. discoideum* led to the production of new metabolites that are currently under investigation. This study shows the huge potential of amoeba as an untapped source of natural compounds and the suitability of *D. discoideum* as a host for the functional expression of polyketide synthases.

### 184-BTP

Exploiting the biotechnological potential of anaerobic fungi

\*M. Stabel<sup>1</sup>, J. Hagemeister<sup>1</sup>, T. Schweitzer<sup>1</sup>, M. Greif<sup>1</sup>, H. Aliyu<sup>1</sup>, K. Ochsenreither<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Institut für Bio- und Lebensmitteltechnik 2, Technische Biologie, Karlsruhe, Germany

The use of lignocellulose containing agricultural waste residues, as wheat straw, for fuel production is one of the prerequisites for a CO<sub>2</sub> neutral economy and society. Anaerobic fungi of the phylum *Neocallimastigomycota* are known lignocellulose degraders and colonizers of ingested

plant material in ruminants and some other herbivores [1]. They have been shown to have the most lignocellulose degrading enzymes inside the fungal kingdom [2]. In addition, they produce valuable metabolites as hydrogen during their mixed acids fermentation [3]. Hence until now their potential remains mainly unexploited. The potential use of *Neocallimastigomycota* for biotechnology consists of two main topics:

1. The exploitation of their already mentioned enzymes for lignocellulose degradation.
2. The direct use of these fungi in fermentation for sugar degradation or metabolite production.

Here we present the heterologous expression of a xylosidase from the anaerobic fungi *Neocallimastix californiae* found by bioinformatic screening and the characterization of its kinetic properties in reactions with 4-Nitrophenylxylopyranosides. In addition, we present first fermentation results from these fungi evaluating metabolite production and C-source consumption.

[1] R. J. Gruninger *et al.*, "Anaerobic fungi (phylum *Neocallimastigomycota*): Advances in understanding their taxonomy, life cycle, ecology, role and biotechnological potential," *FEMS Microbiol. Ecol.*, vol. 90, no. 1, pp. 1–17, 2014.

[2] S. Seppälä, S. E. Wilken, D. Knop, K. V. Solomon, and M. A. O'Malley, "The importance of sourcing enzymes from non-conventional fungi for metabolic engineering and biomass breakdown," *Metabolic Engineering*, vol. 44, pp. 45–59, 2017.

[3] B. Boxma *et al.*, "The anaerobic chytridiomycete fungus *Piromyces* sp. E2 produces ethanol via pyruvate:formate lyase and an alcohol dehydrogenase E," *Mol. Microbiol.*, vol. 51, no. 5, pp. 1389–1399, 2004.

### 185-BTP

Milling by-products are suitable and economical substrates for clostridial ABE fermentation

\*N. Thieme<sup>1</sup>, W. Liebl<sup>1</sup>, V. Zverlov<sup>1</sup>

<sup>1</sup>Technical University Munich, Chair for Microbiology, Freising, Germany

The production of platform chemicals through biorefinery of lignocellulosic plant material was proposed as suitable alternative for oil-based products. Especially *n*-butanol as major product of the clostridial Acetone-Butanol-Ethanol (ABE) fermentation is considered an efficient replacement of gasoline, because of its higher energy density and lower hygroscopicity compared to bio-ethanol. However, the production yield of *n*-butanol is still limited, since the solvent is toxic for the fermenting organisms, and the fermentation process needs further optimization to increase production efficiency and reduce associated costs.

In the present study, we aim to increase the economic viability of ABE fermentation by exploring alternative substrates for ABE fermentation and identifying novel clostridia strains, which exhibit favourable properties for utilization of lignocellulosic plant material and enhanced *n*-butanol production rates. Furthermore, improvements in the efficiency of the fermentation process through the utilization of water-miscible ionic liquids for solvent extraction will be analysed.

Our analyses show, that milling by-products are a suitable substrate for clostridial ABE fermentation. The plant material is produced year-round in large quantities and is of low economic value. However, polysaccharide composition (starch, hemicellulose and cellulose content) and relative abundance make milling by-products, like wheat middlings, suitable substrates for small and medium scale fermentation plants. Our economic evaluation of these approaches showed that annually 20 – 100 kt of wheat middlings could reasonably be utilized for an economically viable ABE fermentation in Bavaria. In addition, a large collection of wild-isolate clostridia strains was screened and revealed nine strains, which exhibited adequate *n*-butanol yields and were able to efficiently utilize milling by-products as substrate. These results indicate that a cost-efficient production of the platform chemical *n*-butanol is possible and that further applications of this solvent, e.g. as lubricant or biofuel, can be developed in the near future.

### 186-BTP

Enrichment and isolation of steroid-hormone degrading bacteria for their use in sewage treatment plants

\*L. Heisel<sup>1</sup>, J. Holert<sup>1</sup>, K. Kujala<sup>2</sup>, B. Philipp<sup>1</sup>

<sup>1</sup>University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany

<sup>2</sup>University of Oulu, Water Resources and Environmental Engineering Research Unit, Oulu, Finland

Steroid sex hormone micropollutants are often not effectively removed from wastewater streams. Residual hormones from wastewater treatment plants entering surface waters have been identified as endocrine disruptors, which calls attention to novel removal strategies. For developing a biological treatment step for eliminating hormonal micropollutants from sewage, androgen- and estrogen-degrading bacteria from activated sludge were enriched and isolated in a continuous flow-through system.

Enrichment conditions were designed for selecting bacteria efficiently removing hormones in micromolar concentrations from wastewater and attaching to specific carrier materials for counteracting cell removal.

The steroid hormones testosterone (TE), estradiol (E2) and ethinylestradiol (EE2) were used in low concentrations as sole carbon sources for bacterial enrichment in 35 ml artificial wastewater medium, which circulated in the system at a 50 ml/min flow rate. The system additionally contained polyethylene particles (PEPs) as biofilm carrier. PEPs were used to transfer cells to the next enrichments. Substrates were quantified by HPLC-MS.

While TE was degraded within 48 h, it took more than 250 h until the estrogen substrates were completely degraded. Enriched cells tended to grow in aggregates attaching to the PEPs.

Upscaling of the system to 1 L did not negatively affect hormone degradation, whereas the addition of acetate and lactate as alternative carbon sources decelerated hormone degradation.

Individual isolates were gained by plating after several enrichment transfers, yielding 2, 4 and 3 strains for TE, E2 and EE2, respectively. All strains were from the genera *Comamonas*, *Methyloversatilis*, *Novosphingobium*, *Pelomonas*, *Pseudomonas* and *Zoogloea*. While all TE isolates were able to degrade TE, estrogen isolates were not able to degrade E2 or EE2, suggesting that mixed cultures are necessary to degrade them.

In conclusion, a continuous flow system with low hormone concentrations is feasible for enriching biofilm-forming hormone degraders from activated sludge. In the next step, these bacteria will be introduced into lab-scale wastewater treatment plants.

### 187-BTP

#### Optimization of Fermentation Parameters in Ethanol Production from Cane Molasses (The case of Metahara Sugar Factory Ethanol Plant)

\*N. Seid<sup>1</sup>, A. Neumann<sup>1</sup>, G. S. Shiferaw<sup>2</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Institute of Process Engineering in Life Science 2, technical Biology, Karlsruhe, Germany

<sup>2</sup>Ababa Institute of Technology, School of Chemical and Bio Engineering, Addis Ababa, Ethiopia

Bioethanol is a high-octane number biofuel which can be produced from the fermentation of starch and sugars. Among the crucial factors affecting bioethanol production, fermentation parameters play significant role on growth of yeast as well as on ethanol yield. The main objective of this study is to optimize the fermentation parameters in ethanol production from cane molasses (the case of Metahara sugar factory ethanol plant). The study has been conducted in such a way that, cane molasses and *schizosaccharomyces pombe* yeast samples collected from Metahara sugar factory ethanol plant and the main composition of cane molasses is analyzed. Then the yeast strain has been cultured in YPD media and cane molasses media. The batch fermentation of molasses samples using *schizosaccharomyces pombe* was carried out at different combinations of fermentation parameters in Erlenmeyer flask of 500 ml. After fermentation of molasses the samples were collected and analyzed for residual sugar concentration, viability of the cell, and ethanol concentration. The experimental design was done by using the Design Expert ® 7.0.0 software five levels; three factors central composite design (CCD) with full type in the optimization study, requiring 76 experiments. To determine the effect of temperature, sugar concentration and fermentation time experiments were done in the ranges of 29 °C to 34 °C, 13% to 22% and 33 hr to 48 hr subsequently. Ethanol yield of 11.04% and residual sugar of 2.6% were obtained under optimum conditions of temperature of 30 °C, sugar concentration of 20% and fermentation time of 45 hr. Validation experiments verified the reliability and the accuracy of the model. These results suggest that to maximize ethanol production in Metahara sugar factory ethanol plant, the optimum fermentation parameters should be control.

### 188-BTP

Establishment of a microfluidic platform using gelmicrodroplets to provide access to the previously unknown microbial diversity

\*B. Kästner<sup>1,2</sup>, P. Neubauer<sup>2</sup>, J. Bader<sup>1,2</sup>, M. Rosenbaum<sup>3</sup>

<sup>1</sup>Beuth University of Applied Sciences, Bioprocess Engineering, Berlin, Germany

<sup>2</sup>Technical University of Berlin, Bioprocess Engineering, Berlin, Germany

<sup>3</sup>Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Bio Pilot Plant, Jena, Germany

Antibiotic resistance is rising to higher levels in all parts of the world, but the development and approval of new antibiotics have been declining over the last decades [1]. Most of the obvious targets for antimicrobial activity have already been discovered. The detection of biosynthetic gene clusters have revealed that many more bacteria than

previously thought are capable to produce metabolites with a potential medical effect [2].

The overarching aim of this project is to identify novel antibiotic producers by accessing the so far uncultured microbial diversity. The project focuses on the establishment of a microfluidic platform forming gel microdroplets (GMDs) suitable for small-scale and high-throughput *in situ* cultivation.

In order to enable growth of the so far uncultured microorganisms, encapsulation at single cell level has to be established. Moreover, approaches to apply gel microdroplets *in situ* to recruit bacteria directly from their natural environment will be explored. The exchange of molecules and thus the uptake of nutrients need to be ensured by the surface of GMDs. Nevertheless, a physical barrier is required to promote the enrichment of slow growing bacteria, which under normal conditions be overgrown by faster ones. Oxygen availability and nutrient supply within the droplet need to be monitored. Furthermore, rheological and physio-chemical parameters play an important role and have to be adjusted for optimal growth conditions.

The encapsulation of single cells in GMDs has been applied in various studies, which aimed for the improvement of the culturability of bacteria from environmental samples [3,4]. However, the implementation of a droplet-based microfluidic platform in high-throughput still remains a challenge. Furthermore, cultivation under close-to nature conditions is another advancement that has to be adapted to GMD techniques with the aim to discover so far hidden microorganisms.

[1] Boucher, H W et al. (2009), Clin Infect Dis., 48(1):1-12.[2] Wohlleben, W et al. (2016), Microb Biotechnol, 9(5): 541-548.[3] Dichosa, A E et al. (2014), Nat Protoc, 9(3): 608.[4] Manome, A et al. (2001), FEMS Microbiol Lett, 197(1):29-33.

## 189-BTP

Heterologous production of the aminoglycoside kasugamycin in *Corynebacterium glutamicum*

\*P. Ramp<sup>1</sup>, S. Matamouros<sup>1</sup>, A. Wirtz<sup>1</sup>, M. Baumgart<sup>1</sup>, M. Bott<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany

**Question** Kasugamycin is an aminoglycoside antibiotic mainly used to control fungal and bacterial crop diseases. It binds between the P- and E-site of the 30S ribosomal subunit in bacteria thereby inhibiting the translation initiation of transcripts containing a 5'-UTR, while leaderless transcripts are unaffected. Leaderless transcripts are highly abundant in *Actinobacteria*, making these organisms very interesting for the production of such an antibiotic. So far kasugamycin is produced by multiple-day fermentation of the natural host *Streptomyces kasugaensis*. Heterologous production was also successful in other actinomycetes, such as *Streptomyces lividans* and *Rhodococcus erythropolis*, but at much lower yields than in the native host. In this study, we attempt to establish heterologous kasugamycin production in the industrially relevant, faster growing actinobacterium *Corynebacterium glutamicum*.

**Methods** The kasugamycin biosynthetic gene cluster containing over 20 different genes was chromosomally integrated into the genome of *C. glutamicum* MB001(DE3) with the positive regulator of the kasugamycin genes set under the control of an inducible promoter. In parallel, adaptive laboratory evolution was performed to identify

beneficial mutations to increase resistance. Analytics were based on a HPLC detection method.

**Results** We report the construction of *C. glutamicum* MB001(DE3)::PT7kas, a strain containing all relevant genes for kasugamycin synthesis. Successful expression of selected genes was confirmed by qRT-PCR. By adaptive laboratory evolution, we isolated strains with increased resistance against kasugamycin. Three mutated genes were identified, one of them being *ksgA* encoding a 16S rRNA adenine methyltransferase, whose deletion is already known to provide kasugamycin resistance. A sensitive and fast HPLC detection method was established allowing the identification of kasugamycin in culture broth supernatants.

**Conclusions** Although, we could confirm expression of several kasugamycin biosynthetic genes in *C. glutamicum*, successful kasugamycin production conditions still need to be established. The kasugamycin resistance mutations will be analyzed further.

## 190-BTP

Lactate utilization in different acetogenic strains

\*F. R. Bengelsdorf<sup>1</sup>, V. Hardt<sup>1</sup>, P. Dürre<sup>2</sup>

<sup>1</sup>Universität Ulm, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany

<sup>2</sup>University of Ulm, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany

Introduction & Objectives:

Lactate is a common substrate for major groups of strictly anaerobic bacteria, and the biochemistry and bioenergetics of lactate oxidation has been described in detail for *Acetobacterium woodii*<sup>1,2</sup>. Besides *A. woodii*, lactate utilization has been mentioned for several other strains including *Butyrivibrio methylotrophicum*, *Clostridium drakei*, *Clostridium scatologenes*, *Clostridium* sp. BPY5, and "Ruminococcaceae bacterium CPB6". Lactate oxidation is mediated by the LDH/Etf complex encoded by the genes *lctBCD* encoded in the *lct* operon. The objective was to study lactate utilization kinetics, product spectrum as well as growth kinetics for *A. woodii* DSM 1030, *B. methylotrophicum* DSM 3468 *Clostridium scatologenes* DSM757.

Materials & Methods:

1. *woodii*, *B. methylotrophicum*, *C. scatologenes* were cultivated anaerobically in either modified ATCC 16123 medium or modified Tanner medium<sup>4</sup> using lactate as substrate. Moreover, the genetic organization of the *lct* operon in different bacterial strains was analyzed using the IMG/ER platform.

Results & Conclusion:

Gene clusters similar to the *lct* operon of *A. woodii* were found in genome sequences of a variety of different anaerobic strains. However, organization of respective genes differs among the analyzed strains. Growth and lactate consumption rates were found to be similar for *A. woodii*, *B. methylotrophicum*, and *C. scatologenes*. The metabolic end product(s) determined for *A. woodii* was acetate, for *B. methylotrophicum* acetate and butyrate, and for *C. scatologenes* acetate, butyrate, and caproate. In conclusion, anaerobic oxidation of lactate can lead to higher value products such as butyrate and caproate or the corresponding alcohols, if bacterial strains are genetically engineered.

## References:

- 1 Weghoff et al., 2015, *Environ Microbiol* 17, 670-677.
- 2 Schoelmerich et al., 2018, *Environ Microbiol* 20, 4587-4595.
- 3 Hoffmeister et al., 2016, *Metab Eng* 36, 37-47.
- 4 Bengelsdorf et al., 2016, *Front Microbiol* 7, 1036.

---

### 191-BTP

#### A Biotechnological Approach towards Mono-functionalized Diamines as Platform Chemicals

\*C. Mügge<sup>1</sup>, A. Gomez Baraibar<sup>1</sup>, A. Maier<sup>1</sup>, S. Wansel<sup>1</sup>, D. Tischler<sup>1</sup>  
<sup>1</sup>Ruhr-Universität Bochum, Bochum, Germany

Aliphatic terminal diamines are a great and versatile basis for functionalization into bulk and fine chemicals. Some of them can easily be obtained from bio-based sources. However, it is extremely difficult to achieve selective monofunctionalization of one amino group by standard synthetic methods. *N*-hydroxylating monooxygenases (NMOs, EC 1.14.13.B10) are a family of flavin-dependent enzymes with the ability to selectively hydroxylate one terminal amino function while keeping the other untouched. The resulting *N*-monohydroxy-diamines can serve as starting components for highly interesting further derivatizations to produce a wide range of fine chemicals.

We aimed at the development of an enzymatic cascade to generate the desired target compounds from  $\omega$ -amino amino acids. For this purpose, the coupling of a suitable amino acid decarboxylase (AAD) with an NMO was pursued. Since NMO requires NADPH to provide the necessary redox equivalents, a regeneration system based on formate dehydrogenase (FDH) also needed to be combined into the enzymatic cascade.

All enzymes were produced in *Escherichia coli* BL21(DE3). NMO from *Gordonia rubripertincta* CWB2 and an FDH mutant capable of providing NADPH was produced in good yields. The enzymatic activities were screened at different conditions (buffer, salt concentration, temperature) using a photometric NADPH assay and a compatibility window of the two processes established. Several AAD were furthermore produced and investigated for their ability to decarboxylate lysine and ornithine with focus on maximum overlap with reaction conditions required for the subsequent step. Towards this aim, a photometric assay based on trinitrobenzene sulfonate was established to quickly identify optimum conditions.

Optimum conditions for each individual enzymatic step were established and a setting suitable for a productive combination of all enzymes was found. All intermediates and products were identified with GC-FID or LC-MS analysis.

A compatibility window for the desired two-step-three-enzyme cascade was established and *N*-monohydroxylated aliphatic diamines as valuable platform chemical produced from bio-based sources.

---

### 192-BTP

#### Immobilized natural compounds of entomopathogenic bacteria on different surfaces inhibit bacterial biofilm formation

\*A. Gazanis<sup>1</sup>, S. Zollner<sup>2</sup>, A. Oelbermann<sup>2</sup>, H. B. Bode<sup>3</sup>, R. Heermann<sup>1</sup>

<sup>1</sup>Johannes Gutenberg-Universität Mainz, Institut für Molekulare Physiologie, Mikrobiologie und Weinforschung, Mainz, Germany

<sup>2</sup>Fraunhofer-Institut für Umwelt-, Sicherheits- und Energietechnik UMSICHT, Materialsysteme und Hochdrucktechnik, Oberhausen, Germany

<sup>3</sup>Goethe-Universität Frankfurt, Fachbereich Biowissenschaften, Molekulare Biotechnologie, Frankfurt am Main, Germany

Bacterial biofilms can not only affect health and environment but also lead to a huge economic loss worldwide. Essential for biofilms to evolve is bacterial communication via small diffusible molecules, also referred to as quorum sensing (QS). Under high cell density, bacteria start to organize in so called biofilms by producing extracellular polymers to attach and grow on nearly every surface. Biofilms are problematic in several sectors such as the shipping industry, health institutes or the drinking water distribution. Entomopathogenic bacteria of the genus *Photorhabdus* and *Xenorhabdus* produce a huge number of novel secondary metabolites. Here we show, that a large number of those natural compounds inhibit biofilm formation without being biocidal. Therefore, we assume that these natural compounds might interfere with QS of the target bacteria to block their communication, a process that is referred to as quorum quenching (QQ). We observed, that bacteria of equal biofilm habitats exhibited similar susceptibility towards the same QQ-compounds. Interestingly, three of the tested compound classes had a severe effect also at very low concentrations. A self-constructed microfluidic flow cell gave us the opportunity to analyze the structure and composition of different bacterial biofilm communities via microscopy on the one hand and visualize the effect of the natural compounds on the other hand. We were then able to coat different materials with the compounds and immobilize those on the respective surface. In order to simulate more realistic native conditions, we constructed another specific microfluidic cell, placed the coated material inside and analyzed bacterial attachment under constant flow. These coated materials were used to test the effect of the natural compounds in native environments e.g. water pipes, ship hulls and harbors. In summary, our data indicate that entomopathogenic bacteria represent an excellent reservoir of QQ-compounds that are putatively applicable as specific biofilm preventive agents.

---

### 193-BTP

The author has not agreed to a publication.

---

### 194-BTP

#### Metagenomic nanopore sequencing for the characterization and abundance tracking of complex microbial communities in biogas reactors

\*C. Brandt<sup>1,2</sup>, E. Bongcam-Rudloff<sup>2</sup>, B. Müller<sup>3</sup>

<sup>1</sup>Jena University Hospital, Institute for Infectious Diseases and Infection Control, Jena, Germany

<sup>2</sup>Swedish University of Agricultural Sciences, Dept. Animal Breeding and Genetics, Bioinformatics section, Uppsala, Sweden

<sup>3</sup>Swedish University of Agricultural Sciences, Department of Molecular Sciences, Uppsala, Germany

## Introduction

Anaerobic digestion (AD) has been a critical technology for green energy for many decades. However, the majority of the microorganisms involved are unknown and not cultivable, which makes abundance tracking difficult. The accessibility

of Nanopore (ONT) sequencing may provide a tempting alternative to monitor the microbial community via metagenomic sequencing. Although long reads are less accurate, the higher information richness may allow for a similar specific taxonomic classification, e.g., via Illumina. Additionally, the sequencing yield still reflects the absolute abundance of an organism.

For reliable monitoring of AD via long reads, a robust protocol for less fragmented and high-quality DNA, while preserving bacterial composition needs to be established. Additionally, identifying the most suitable index database for the best overall classification performance is crucial.

## Materials & methods

We investigated 20 different biogas/waste-water reactors (DNA isolation protocol available at [dx.doi.org/10.17504/protocols.io.w7afhie](https://dx.doi.org/10.17504/protocols.io.w7afhie)). All samples were subject to Nanopore and Illumina sequencing. Read classification was performed using Centrifuge. Different Index databases were built from RefSeq and GTDB to compare read classification performance.

## Results:

We retrieved a median of 20 Gb sequencing data per flow cell. Taxonomic classification achieved a dramatic classification increase when using an Index build from GTDB compared to the RefSeq index. The improvement of classified reads ranged between 1.8 to 13-fold increase. Moreover, phyla estimations via GTDB are in line with 16S studies and MAG studies pointing to Firmicutes and Bacteroidetes as the main phyla for most AD"s.

## Conclusion

Long-read sequencing via Nanopore provides a cheaper and more accessible alternative for taxonomic read classification. The ongoing effort on GTDB for more phylogenetically-coherent taxonomic species definitions incl. meta-assembled genomes have a clear advantage over traditional classification databases such as RefSeq. Compared to conventional 16S studies, Metagenomic read classification allows us to monitor the abundance of the unknown microbial fraction.

### 195-BTP

#### **Cupriavidus necator as versatile biocatalyst powered by electrochemically generated H<sub>2</sub>**

\*H. Wohlers<sup>1</sup>, D. Holtmann<sup>1,2</sup>

<sup>1</sup>DECHEMA Forschungsinstitut, Industrial Biotechnology, Frankfurt am Main, Germany

<sup>2</sup>Technische Hochschule Mittelhessen, Life Science Engineering, Gießen, Germany

*Cupriavidus necator* is a gram-negative bacterium which is able to grow chemolithoautotrophically. During lithoautotrophic growth it fixes carbon dioxide using hydrogen as an electron donor and oxygen as an electron acceptor. *C. necator* harbours oxygen-tolerant hydrogenases which allow the use of hydrogen as a cheap reducing agent for cofactor regeneration in an aerobic whole cell bioprocess. Therefore, *C. necator* is a promising platform for whole cell biotransformations using oxygen-requiring, NAD(P)H dependent enzymes, such as cytochrome p450 monooxygenases and ene-reductases Both enzyme classes are of great interest for the synthesis of fine chemicals since they provide the

opportunity to overcome the obstacles of classic chemical synthesis concerning the activation of inert carbohydrates. For instance P450 BM-3 (CYP102A1) from *Bacillus megaterium* is a soluble, catalytically self-sufficient monooxygenase which is able to catalyze regio- and stereospecific hydroxylations of non-activated carbon-hydrogen bonds by inserting one oxygen atom from molecular oxygen into the substrate and the ene-reductase YqjM from *Bacillus subtilis* is a flavoprotein that can catalyze the trans-hydrogenation of conjugated C=C bonds.

The successful expression of the P450 monooxygenase (unpublished work) and the ene-reductase<sup>1</sup> in *C. necator* as well as its growth inside a bioelectrochemical system<sup>2</sup> has already been shown. Here we present the coupling of the enzyme expression with the electrobiochemical cultivation of *C. necator*. This system will provide the opportunity for chemical storage of surplus energy from renewable sources while producing chemicals of high value.

1.Assil-Companiononi, L., Schmidt, S., Heidinger, P., Schwab, H. & Kourist, R. Hydrogen-driven cofactor regeneration for stereoselective whole-cell C=C bond reduction in *Cupriavidus necator*. ChemSusChem cssc.201900327 (2019). doi:10.1002/cssc.201900327

2.Krieg, T., Sydow, A., Faust, S., Huth, I. & Holtmann, D. CO<sub>2</sub> to Terpenes: Autotrophic and Electroautotrophic  $\alpha$ -Humulene Production with *Cupriavidus necator*. Angew. Chemie - Int. Ed. 57, 1879–1882 (2018).

### 196-BTP

Biofilm formation in CSTR biogas reactors at very low hydraulic retention times

\*D. Popp<sup>1</sup>, A. Weimer<sup>1,2</sup>, T. R. Neu<sup>3</sup>, F. Centler<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Saarland University, Institute for Systems Biotechnology, Saarbrücken, Germany

<sup>3</sup>Helmholtz Centre for Environmental Research - UFZ, River Ecology, Magdeburg, Germany

Anaerobic digestion is an efficient biotechnological process by which agricultural, municipal, and industrial organic waste can be converted into biogas which is utilized as electricity, heat, and fuel. One important process parameter of biogas reactors is the hydraulic retention time (HRT) which is adjusted as a compromise between degradation efficiency and reactor size – both crucial for the economics of biogas production. In this study, the HRT of duplicate, lab-scale continuous stirred tank reactors (CSTR) digesting volatile fatty acids (acetic, propionic, and butyric acid) was systematically lowered starting from 8 d. A third reactor was operated as control constantly at the initial HRT.

Efficient biogas production was still observed at 3.9 d HRT and an organic loading rate of 7 g VFA L<sup>-1</sup> d<sup>-1</sup> for both reactors. Process breakdowns due to accumulation of volatile fatty acids occurred at 2.7 d and 1.9 d HRT for the first and the second reactor, respectively. Differences between duplicate reactors at the fatal HRTs were also observed regarding the microbial communities as analyzed by amplicon sequencing of 16S rRNA and *mcrA* genes. In the first reactor *Methanosaeta* was the dominant methanogen (72% relative abundance) which is solely able to produce methane from acetate. Furthermore, the hydrogenotrophic methanogens *Methanoculleus* (25%) and *Methanospirillum* (2%) were found. In the second reactor the

methanogen *Methanosarcina* was increasingly detected with lower HRTs with up to 31% relative abundance. Biofilms formed mainly at the bottom of the reactors for both experimental and control reactors. These biofilms were also dominated by *Methanosaeta*. The dominance of *Methanosaeta* was consistent with the filamentous cells dominantly present in the biofilm as revealed by confocal laser scanning microscopy.

Biofilm formation in CSTRs might be a microbial survival strategy to prevent washout at very low HRT. Even though CSTR reactors are not intended to retain biomass, enforced biofilm formation by an adapted reactor design might present a promising option to further optimize biogas production in such reactors.

---

### 197-BTP

Isolation of a new *Streptomonospora*-species producing a new peptide antibiotic

\*S. Khodamoradi<sup>1</sup>, R. Hahnke<sup>1</sup>, M. Stadler<sup>1</sup>, F. Surup<sup>1</sup>, J. Wink<sup>1</sup>, P. Schumann<sup>1</sup>, M. Steinert<sup>1</sup>, M. Rohde<sup>1</sup>

<sup>1</sup>Helmholtz center for infection research, Microbial group collection, Braunschweig, Germany

Most important antimicrobial drug classes, such as tetracyclines, rifamycins, aminoglycosides, macrolides, beta-lactams, and glycopeptides are produced by actinomycetes. However, more and more pathogenic bacteria show resistances against these common antibiotics. Today, one of the major challenges is to treat infection diseases, caused by (multi-) drug-resistant strains. New antibiotics have to be created synthetically or found by screening natural resources from the environment. Especially new species, genera and families of actinobacteria harbour a high potential to produce new antibiotics.

Within the search for new antibiotic producing actinobacteria, two strains of rare actinomycetes were isolated from the coastal sand (Cuxhaven beach/ Germany). The strains are closely related to each other, are Gram-positive, rod shape, non-motile, spore forming and exhibit extensively branched substrate mycelia and aerial hyphae. They will be described as members of a new species within the genus *Streptomonospora*. Based on 16S rRNA gene sequence the closest strain is *S. halophila* DSM 45075 with (98%) similarity. Crude extracts showed interesting antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus lutes*, *Clostridium difficile*). After optimization, fermentation, HPLC and mass spectrometry analysis, the accurate mass of the active compound was determined and proposed as a new derivation of a peptide antibiotic class. Next steps will be optimization of production media, scale-up, structure elucidation with NMR and bioactivity spectrum against a large panel of test (pathogenic) organisms to determine the MIC-values of the new antibiotic.

---

### 198-BTP

Evaluation of "Impure" Syngas derived from Biomass Gasification as Substrate in Syngas Fermentation

\*A. Infantes<sup>1</sup>, A. Neumann<sup>1</sup>

<sup>1</sup>KIT (Karlsruher Institut für Technologie), Institute of Process Engineering in Life Sciences, Section II: Technical Biology, Karlsruhe, Germany

### Introduction

In this study, the effect of various biomass-derived syngas bottled directly at the gasification site on a *Clostridium ljungdahlii* fermentation process has been evaluated.

Acetogenic microorganisms, including *C. ljungdahlii*, represent a promising platform to advance towards the production of renewable, environmentally friendly chemical building blocks and commodity chemicals, as opposed to the current ones, mainly fossil-fuel based.

As opposed to traditional chemical catalysts, this group of microorganism can tolerate some impurities that are present in the syngas as well as being able to use various mixtures of CO and/or CO<sub>2</sub> and H<sub>2</sub>. These features make them an attractive alternative to chemical processes.

The typically expected impurities in the "real" syngas are: methane (CH<sub>4</sub>), ethane (C<sub>2</sub>H<sub>6</sub>), ethylene (C<sub>2</sub>H<sub>4</sub>), acetylene (C<sub>2</sub>H<sub>2</sub>), tar components like benzene, and hydrogen sulphide (H<sub>2</sub>S). Moreover, traces of other C<sub>3</sub>, C<sub>4</sub>, C<sub>5</sub> and C<sub>6</sub> components can be present. The exact quantities and type of impurities will variegate depending on the syngas source, due to the intrinsic natural variability of the biomass used in the gasification process.

### Objectives

Most of the research performed to date has used pure syngas, containing only a defined composition of CO and/or CO<sub>2</sub> and H<sub>2</sub>. Here, we analyse how the impurities, or the combined effect of the components in the "real" syngas, which is derived directly from the gasification of biomass, affects the outcome of the process, in comparison to pure syngas.

### Methods

The effects of the different gases compositions was exhaustively monitored on a well-established, robust fermentation system with continuous monitoring of the gas consumption via GC.

5 batch fermentations were done in triplicate with *C. ljungdahlii*, using gases from 4 different gasification plants. Gas consumption, growth and products were analysed.

Moreover, 3 synthetic gas mixtures, with the same CO, CO<sub>2</sub> and H<sub>2</sub> composition as the "real" syngas here tested, but without the impurities, were also fermented in order to clearly assess the impurities' effect.

The results are being analysed and will be presented here.

---

### 199-BTP

A sugar-independently regulated gene expression system for *Pseudomonas fluorescens*

\*L. Hoffmann<sup>1</sup>, T. Brüser<sup>1</sup>

<sup>1</sup>Leibniz Universität Hannover, Institut für Mikrobiologie, Hannover, Germany

Species of the genus *Pseudomonas* are important organisms for medical and environmental research. Studies that analyze these organisms often use tools of molecular microbiology, such as targeted mutagenesis and genetic complementation. There are only sugar-regulated promoter systems established for the recombinant expression of genes in pseudomonads. As sugars themselves can strongly influence metabolic pathways in possibly non-desired ways,

we sought after a promoter that could permit the tightly regulated gene expression in a sugar-independent manner. We therefore screened the genome of our model organism *P. fluorescens* strain A506 for likely AraC-family regulators that are encoded in direct vicinity of an assigned metabolic operon unrelated to sugar metabolism. Among the identified regulators, the gene PflA506-4486 encodes for a putative anthranilic acid inducible regulator, as this gene is located directly upstream of the *antABC* operon for anthranilic acid degradation to catechol. The homolog of this putative regulator has been shown to activate *antABC* expression in the related pathogen *P. aeruginosa* [1]. We thus cloned PflA506-4486 and the putative promoter region for *antABC* expression, and used the gene for mCherry as reporter to examine promoter activity. For comparison, we constructed the accordant reporter system also for the standard rhamnose-inducible promoter. The direct comparison of the two expression systems is shown.

[1] Choi et al. (2011) Growth phase-differential quorum sensing regulation of anthranilate metabolism in *Pseudomonas aeruginosa*. *Mol. Cells* **32**, 57-65

## 200-BTP

A new carbonyl reductase from *Yarrowia lipolytica*

stereospecifically converting  $\alpha$ -substituted  $\beta$ -keto esters

\*C. Drewniak<sup>1</sup>, C. Trapp<sup>1</sup>, C. Schuster<sup>1</sup>, K. Barková<sup>1</sup>, N. Jehmlich<sup>2</sup>, D. Greif<sup>1</sup>, A. Fuchs<sup>1</sup>, T. Wiegert<sup>1</sup>, M. J. Pecyna<sup>1</sup>

<sup>1</sup>Zittau/Goerlitz University of Applied Sciences, Faculty of Natural and Environmental Sciences, Zittau, Germany

<sup>2</sup>Helmholtz-Centre for Environmental Research - UFZ, Department of Molecular Systems Biology, Leipzig, Germany

For the synthesis of novel chiral building blocks, e.g. Evans auxiliaries, suitable biocatalysts for stereospecific reductions of  $\alpha$ -substituted  $\beta$ -keto-esters are of major interest. Therefore, different yeast strains were analyzed in whole cell biotransformation assays for the reduction of ethyl acetoacetate (EAA) and prochiral hydrophobic derivatives of EAA. Reaction products were analyzed by HPLC and GC. *Yarrowia lipolytica* DSMZ 3286 was able to reduce ethyl 2-benzylacetoacetate with an excess of the *anti* diastereomer product (de > 99%). A respective NADPH-dependent carbonyl reductase with a molecular mass of 96.7 kDa could be purified to homogeneity from wild-type cells of *Y. lipolytica*. The protein was identified by peptide-mass-fingerprinting (nanoLC / ESI-MS of tryptic peptides with 94% coverage) as a member of the short-chain dehydrogenase/reductase protein family. Its gene was expressed in *Escherichia coli* fused to a hexahistidine-SUMO-tag and proved to be active for the reduction of ethyl 2-benzylacetoacetate as a substrate. Kinetic properties, substrate specificities and stereoselectivity of the enzyme will be presented.

## 201-BTP

Robot-based automated isolation and characterization of a colicin-producing *Escherichia coli* strain with inhibitory activity against EPEC

\*B. Heilmann<sup>1</sup>, S. Özipek<sup>2</sup>, L. Stähelin<sup>1</sup>, M. Machava<sup>1</sup>, P. Götz<sup>1</sup>, J. Bader<sup>1</sup>, E. Grohmann<sup>3</sup>

<sup>1</sup>Beuth University of Applied Sciences, Bioprocess Engineering, Berlin, Germany

<sup>2</sup>Free University of Berlin, Biology, Chemistry, Pharmacy, Berlin, Germany

<sup>3</sup>Beuth University of Applied Sciences, Microbiology, Berlin, Germany

Since antibiotic resistance is growing at alarming rates, causing a global health threat, there is an urgent need for the development of innovative strategies for the discovery of new antibiotic substances. While former strategies for antimicrobial drug discovery mainly focused on synthetic chemicals, modern approaches are now returning to the investigation of natural resources [1]. Recently, colicins are regaining attention as potential alternatives to traditional antibiotics [2]. Colicins are antimicrobial proteins produced by *Escherichia coli* (*E. coli*) strains that inhibit or kill closely related bacteria with high specificity.

To approach this urgent task, we aim to establish a laboratory for the isolation and production of new antimicrobial agents from natural products. State-of-the-art technology is used to design an advanced robot-based discovery platform, with a specific focus on automated high throughput screenings (HTS) for the isolation of microorganisms from environmental samples as well as for antimicrobial activity testing, purification of the substance and fermentation process development.

A small-scale robot-based HTS was conducted to isolate bacteria with antagonistic activity against human pathogenic bacteria from sludge. The screening revealed a promising candidate, identified by MALDI Biotyper System and 16S rRNA gene sequencing as a non-pathogenic *E. coli*. The *E. coli* showed antimicrobial activity against two enteropathogenic *E. coli* (EPEC) strains. While a PCR-analysis revealed the existence of three genes encoding the colicins B, E1 and M, analyses by SDS-overlay assays indicated that only colicins E1 and M are effectively inhibiting cell growth of the EPEC strains. Further analysis is focusing on the development of a novel purification strategy for colicins and the optimization of the induction of colicin synthesis.

Further steps will comprise the optimization of colicin purification by establishing a small-scale robot-based automated purification unit as well as the improvement of the fermentation process.

[1] Brown, ED & Wright GD (2016) *Nature*, 529, 336-343

[2] Cotter, PD et al. (2012) *Nature Reviews Microbiology*, 11, 95-105

## 202-BTP

Proteolytic enzymes of *Aspergillus* with activity in relation to components of the hemostasis system for diagnosis of thromboembolic diseases

\*A. Osmolovskiy<sup>1</sup>, A. Orekhova<sup>2</sup>, A. Ochneva<sup>2</sup>

<sup>1</sup>Moscow State University, Microbiology, Moscow, Russian Federation

<sup>2</sup>Lomonosov Moscow State University, Microbiology, Moscow, Russian Federation

Huge interest for practical medicine have proteinases with the ability to activate some of the hemostatic system proteins by their limited proteolysis. Fungi from *Aspergillus* genus are able to secrete proteases, activating protein C and factor X - core proteins of the hemostatic system, changing the content of which in the blood stream leads to various diseases. Such enzymes are highly specific to these target proteins and may prove to be very promising for the development of drugs and diagnostic products to replace the existing foreign counterparts, mainly of animal origin.

Aim of the research was study of biotechnological potential of some *Aspergillus* fungi for production proteases for determination concentration of protein C and factor X in human plasma.

*Aspergilli* were cultivated under submerged conditions, protease activity detection was made using chromogenic peptide substrates specific for activated protein C (S-2366) and factor Xa (S-2765) after the addition of blood plasma (activator activity).

10 strains from *Aspergillus* genus were tested for the ability to produced proteases with activating activity toward protein C and factor X of human plasma. Proteases of *A. ochraceus* and *A. sclerotiorum* were promising as producers of activators target enzymes. Their properties are similar to proteinases-activators derived from the venom of the southern American copperhead snake and Russell's viper, respectively, and which used for the diagnosis of these proteins is currently. The character of the calibration curves for determination in plasma protein C and factor X levels in case of usage proteinase of *A. ochraceus* is very close to the character of commercial analogs" schedules (based on snake venom proteases). Applicability of the calibration curves was checked by suitable reactions with plasmas with artificially reduced level of protein C and factor X or deficient plasmas. It was shown that the level of their content reliably is determined using of proteases of *A. ochraceus*.

So, that micromycetes extracellular proteases of *A. ochraceus* and *A. sclerotiorum* can be used for mesuaring of the amount of protein C and factor X in human plasma.

---

### 203-BTP

#### How much can you bear? - Alcohol tolerance in microbial expression strains

\*F. Schultes<sup>1</sup>, M. Haarmann<sup>1</sup>, D. Tischler<sup>1</sup>, C. Mügge<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Mikrobielle Biotechnologie, Bochum, Germany

Alcohols, as well as many other organic solvents, are mostly toxic to microorganisms. Due to the rising demand for environmentally friendlier ways to produce chemicals, the need for biocatalysts, expression organisms and whole cell bioconversions has increased. During bioconversion processes, alcohols can be used as both, substrates and solvents. Taking this, we aim to test *Pseudomonas putida* KT2440 and *Escherichia coli* (*E. coli*) expression strains for their tolerance towards short to middle length carbon chain alcohols.

For testing these tolerances, the different expression organisms/strains were subjected to rising concentrations of alcohol with carbon chain lengths from C1 to C8 until no growth was observed and cell death occurred. Due to different deletions within typical expression strains, alcohol tolerance can differ significantly. To determine if gene knocked outs helped the tolerance or leveled the sensibility towards alcohols, single knock out strains were investigated to check if and what impact a certain knock out has on alcohol tolerance.

Results showed that *E. coli* BI21 (DE3), *E. coli* Top10 and *E. coli* BW25141 have slightly different tolerances towards hexanol, which is also the most toxic alcohol tested. The other alcohols tested were far less toxic for the cells. In comparison to this, *Pseudomonas putida* KT2440 showed a relatively high tolerance towards hexanol but was very sensible towards the other alcohols with a very low threshold.

There are substantial differences in alcohol tolerance and sensibility within the investigated strains, depending on the knockout pattern or intrinsic capabilities to cope with certain substances. Further tests should reveal different strain capabilities as well as function on sensibility of single knock out mutants and enable us to determine which strain to use for specific bioconversions.

---

### 204-BTP

#### Enriching the diversity of polyethylene terephthalate (PET) degrading enzymes from metagenomes

\*H. Zhang<sup>1</sup>, D. Danso<sup>1</sup>, J. Chow<sup>1</sup>, B. Voss<sup>2</sup>, J. Pleiss<sup>3</sup>, W. R. Streit<sup>1</sup>

<sup>1</sup>University of Hamburg, Institute of Plant Science and Microbiology, Germany, Hamburg, Germany

<sup>2</sup>University of Stuttgart, Biochemical Engineering - Computational Biology, Stuttgart, Germany

<sup>3</sup>University of Stuttgart, Biochemistry and Technical Biochemistry, Stuttgart, Germany

**Introduction:** Polyethylene terephthalate is a durable and strong, chemically and thermally stable polyester. Due to these desirable properties, PET is used widely for a variety of applications in industry. However, PET plastics do not degrade to a large degree when released into the environment (1). Its accumulation has become one of the major concerns of plastic pollution. Compared to traditional PET recycling processes requiring lots of energy resources and usually produce harmful contaminants, the use of microorganism-derived enzymes has provided a novel environmentally friendly option for the recycling of PET materials.

**Objective:** This project mainly focus on the isolation and characterization of the novel potential enzymes that have the ability to degrade PET. We combine enrichment cultivations and metagenomics to identify enzymes with activity on this persistent polymer.

**Methods:** The environmental samples were collected from an area in Wietze (Lower Saxony, Germany), where soils are heavily contaminated by crude oil. To obtain potential Enzymes that can degrade PET, the enrichment cultivations, metagenome sequencing, bioinformatics analysis, heterologous and *in vitro* -expression combined with functional test have been employed.

**Results:** Four consortiums with ability to degrade plastics were isolated from the environmental samples. Based on metagenome sequences and hmm searches, we have been synthesizing 30 potential PET hydrolases found by a PETase-specific hidden Markov model (2). By using combined bioinformatics analysis and functional test methods, we were able to identify at least 15 novel PET hydrolases from different bacterial phyla and have expand the diversity of these enzymes significantly.

(1) Danso D. *et al.*, (2018), DOI: 10.1128/AEM.02773-17

(2) Danso D., Chow J., Streit W.R. (2019), DOI: 10.1128/AEM.01095-19

---

### 205-BTP

#### Analysis of regulatable heterologous expression systems in *Gluconobacter oxydans* 621H

\*P. M. Fricke<sup>1</sup>, C. Davoudi<sup>1</sup>, T. Link<sup>1</sup>, C. Sonntag<sup>1</sup>, L. Flachbart<sup>1</sup>, M. Bott<sup>1</sup>, T. Polen<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich GmbH, Institut für Bio- und Geowissenschaften IBG-1: Biotechnologie, Jülich, Germany



**I.:** *Gluconobacter oxydans* 621H is an acetic acid bacterium known for its ability to incompletely oxidize numerous carbohydrates in the periplasm by membrane-bound dehydrogenases (mDHs). Industrial applications make use of *G. oxydans*" many mDHs that provide a regio- and stereospecific conversion of a broad range of substrates. Also, the heterologous expression of uncharacterized mDHs is interesting for their analysis. However, hitherto no inducible expression system with a high dynamic range could be established in *G. oxydans*.

**O.:** Here we analyzed in *G. oxydans* 621H the performance of heterologous expression systems well-established in bacteria other than acetic acid bacteria.

**M.:** *G. oxydans* expression plasmids harboring *eyfp*, *mNeonGreen*, or *uidA* as reporter genes were constructed based on pBBR1. Recombinant *G. oxydans* strains were analyzed for growth, reporter expression, and cell heterogeneity using Biolector, FACS, DNA microarray, and LC-MS/MS-based proteomic analysis as well as UidA enzyme assays.

**R.:** Heterologous LacI-controlled *eyfp* expression required high IPTG concentrations (10 mM) and reached only a 7-fold increase in fluorescence. When using genomically integrated and IPTG-inducible T7 RNA polymerase and T7 promoter-controlled *eyfp* expression, induction reached only a 3-fold increase. In the TetR-dependent expression system, a maximal induction of 11-fold was determined. DNA microarray analysis revealed very high *eyfp* reporter mRNA levels already in non-induced samples, yet only low eYFP fluorescence was measured which peaked after 8 h and then rapidly decreased by ~50%. Both, LacI and TetR repressor proteins were present according to LC-MS/MS results.

**C.:** The performance of inducible expression systems well-established in several bacteria is low in *G. oxydans*. In spite the presence on the protein level repressor proteins did not sufficiently repress reporter gene expression. Also, the apparent decrease of the reporter signal/activity remains unclear.

---

## 206-BTP

The author has not agreed to a publication.

---

## 207-BTP

Utilizing cyanobacterial photosynthesis as power supply for whole-cell-biocatalysis

\*A. Hochkeppel<sup>1</sup>, J. Toepel<sup>1</sup>, M. Uhl<sup>1</sup>, A. Hoschek<sup>1</sup>, B. Bühler<sup>1</sup>, A. Schmid<sup>1</sup>

<sup>1</sup>Helmholtz-Zentrum für Umweltforschung (UFZ) Leipzig, Solar Materials, Leipzig, Germany

Cyanobacteria are promising microbial hosts for sustainable biotechnological production processes. They are photosynthetically active and relatively easy to genetically engineer, two features paving the way for their use in photo-biotechnology. The model organism *Synechocystis* sp. PCC6803 has been used to connect different reactions to the photosynthetic light reaction [1],[2]. Photosynthesis-derived oxygen and reduction equivalents make the linkage of oxygenation reactions to photosynthesis particularly interesting, as these reactions require oxygen and reduction equivalents. We engineered *Synechocystis* sp. PCC6803 with a Baeyer-Villiger-Monooxygenase (BVMO) from *Acidovorax* sp. CHX100 catalyzing the conversion from Cyclohexanone to  $\epsilon$ -Caprolactone, a precursor for biopolymer synthesis[3]. Two strategies were used: a)

Genome-integration of the BVMO gene and b) episomal expression of BVMO from a pPMQAK1 based plasmids. Genome-based expression of BVMO led to remarkable  $\epsilon$ -Caprolactone production with a specific activity up to 33.0 U gCDW<sup>-1</sup>. Besides side-reactions, the low BVMO expression is suspected the main hindrance for a more efficient biotransformation. Our studies show *Synechocystis* sp. PCC6803 being an attractive whole-cell-biocatalyst for photo-biotechnological production processes, as  $\epsilon$ -Caprolactone formation. Future research objectives are the further development of molecular tools to precisely engineer cyanobacteria for biotechnology application. Furthermore, a more in-depth analysis of the metabolism of photosynthetically active cell will help to establish photo-biotechnology in *Synechocystis* sp. PCC6803.

## References:

[1] Böhmer, et al. (2017)

[2] Hoschek, et al. (2019)

[3] Karande, et al. (2018)

---

## 208-BTP

### Electrode assisted production of platform chemicals in *R. sphaeroides*

\*F. Schmid<sup>1</sup>, J. Gescher<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Institute for Applied Biosciences (IAB) - Department of Applied Biology, Karlsruhe, Germany

**Introduction:** The aim of this study is using methods of synthetic biology to supply bacterial production strains with reducing power directly through electrical energy. By using the provided reducing power, highly oxidized substances as CO<sub>2</sub> can be used to synthesize reduced platform chemicals.

**Objectives:** The photosynthetic non sulfur purple bacterium *Rhodospirillum rubrum* is cultivated in a bioelectrical system (BES) in which light, CO<sub>2</sub> and electrical current is provided. By fixing CO<sub>2</sub> via anoxygenic photosynthesis, acetoin should be produced via the heterologous expression of the corresponding metabolic pathway. By introducing electron transporting c-type cytochromes in *R. sphaeroides* a direct microbe electrode interaction should be established. This would change the photolithoautotrophic to a photoelectroautotrophic lifestyle.

**Methods:** The BES is operated by a potentiostat running a chronopotentiometry, in which the electrochemical potential necessary to maintain a set current is measured. Afterwards the biofilm formation on the cathode is quantified (total protein / total DNA per cm<sup>2</sup>) and analysed by microscopy (DAPI, Live/dead staining) to investigate the formation of biofilm. The concentration of acetoin is measured with the voges-proskauer test.

**Results:** There is evidence that cultivation of *R. sphaeroides* mutants which express cytochromes shows a less negative potential needed to maintain a set current than cultivation of *R. sphaeroides* without c-type cytochromes. This could be a result of the electrons being transferred directly to the cell instead of forming H<sub>2</sub> which is then oxidized by *R. sphaeroides*. The presence of cytochromes enhances biofilm formation on the cathode. Also acetoin can be produced in *R. sphaeroides*.

**Conclusion:** There are hints for a direct microbe cathode interaction which is mediated by c-type cytochromes.

Experiments for further investigation on the exact nature of this interaction will be performed. This work is a step towards the photoelectroautotrophical production of acetoin in *R. sphaeroides*.

---

## 209-BTP

Real-time monitoring of fungal growth on solid substrates

\*H. L. Duong<sup>1</sup>, T. Maskow<sup>1</sup>, D. Schlosser<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany

Fungal solid-state fermentation (SSF) is useful for different biotechnological applications such as lignocellulose pre-treatment for biorefinery approaches, composting, mushroom production, and certain bioremediation schemes. Indirect methods for monitoring fungal growth during SSF are commonly laborious and time consuming.

We therefore aimed to investigate whether fungal growth could be sufficiently recorded using an alternative direct method, i.e. real-time biocalorimetry targeting the metabolic heat evolution. For this purpose, we recorded the metabolic heat production rate generated by the ubiquitous ascomycetous mold *Stachybotrys chlorohalonata* during growth on a solid lignocellulosic substrate (wheat straw). Fungal ergosterol determination was additionally applied as a conventional indirect method for fungal biomass estimation, and correlated with biocalorimetric data.

The biocalorimetric measurements demonstrated that the growth-related metabolic activity of *S. chlorohalonata* rapidly increased during the initial phase of growth and was highest already at culture day 6. This pattern is in accordance with an R-selected fungus, which colonizes new resources and preferentially degrades easily available compounds while rapidly collapsing in response to nutrient depletion. Fungal biomass as determined by ergosterol determination exhibited a strong linear correlation with the integral of the heat production rate (Pearson product-moment correlation coefficient > 0.97).

Our results prove biocalorimetry suitable for the rapid and non-destructive real-time monitoring of fungal growth during colonization of solid substrates.

---

## 210-BTP

**Towards construction of *C. acetobutylicum* ATCC824 and *C. saccharobutylicum* NCP262 strains for efficient conversion of milling by-products in butanol.**

\*H. Edelmann<sup>1</sup>, J. Müller<sup>1</sup>, C. Pires<sup>2</sup>, N. Saunders<sup>2</sup>, W. Liebl<sup>1</sup>, A. Ehrenreich<sup>1</sup>

<sup>1</sup>Technical University Munich, Mikrobiologie, Freising, Germany

<sup>2</sup>Brunel University London, Systems and Synthetic Biology, Uxbridge, United Kingdom

The acetone-butanol-ethanol (ABE) fermentation of lignocellulosic waste materials by *Clostridia* would be a sustainable and CO<sub>2</sub> neutral alternative for the production of butanol. Recent developments in fermentation and product separation techniques, as well as construction of genetically optimized production strains make this process an alternative to petroleum-based butanol.

In this study, we use genetically optimized clostridia strains for the production of butanol from milling by-products. These substrates contain sizable amounts of starch but are also rich in hemicelluloses. The problem in the usage of this substrate is the catabolite repression of the utilization of the

pentoses xylose, arabinose and the hexoses mannose and galactose resulting from hemicellulose degradation by the glucose from starch. This results in ineffective overall utilization of the whole complex substrate.

In order to solve this problem, we generated mutant strains of *C. acetobutylicum* ATCC 824 with altered carbon catabolite repression (CCR) in continuous cultures. Sites of mutations of the most efficient mutants, able to metabolize various hexoses and pentoses in the presence of glucose where characterized by whole genome sequencing. The mutations are located in the glucose- and mannose-specific phosphotransferase systems as well as in a gene coding for a sigma factor of the SigD/WhiG family. Besides faster sugar uptake, one group of CCR-deficient mutants showed a threefold higher butanol yield in batch fermentation on straw hydrolysate than the wild-type. This was accompanied by a complete reabsorption of butyric acid. A marker-less genetic system established for *C. saccharobutylicum* is adapted to *C. acetobutylicum* in order to combine the mutations for most effective sugar utilization with heterologous expression and secretion of hemicellulases in order to improve breakdown efficiency of the complex substrate. Similar work is done on *C. saccharobutylicum* NCP262 in order to construct efficient solvent forming strains also from this organism.

---

## 211-BTP

Identification and evaluation of novel subtilases from *Bacillaceae*

\*F. Falkenberg<sup>1</sup>, J. Rahba<sup>1</sup>, P. Siegert<sup>1</sup>, J. Bongaerts<sup>1</sup>

<sup>1</sup>FH Aachen-University of Applied Sciences, Campus Jülich, Institute of Nano- and Biotechnologies, Jülich, Germany

Proteases have long been used to catalyze the hydrolysis of peptide bonds in aqueous solutions. Especially extracellular alkaline proteases of microbial origin are well established in many technical applications, e.g. as ingredients in household laundry detergents worldwide, where they have been a component since 1985 (Coker 2016). Proteases make up about 60% of the total world enzyme production (Kalisz 1988; Maurer 2004). Due to the high demand of technical enzymes on the global market, there is still a need for novel and improved molecules. Most commercially used alkaline proteases are based on subtilisin backbone (Maurer 2004, Siezen and Leunissen 1997). In order to broaden the sequence space of bacterial alkaline proteases from subtilisin to subtilase family, a systematic database analysis was performed. Besides the genus bacillus, a specific focus was placed on further genera of the family bacillaceae. A set of new uncharacterized protease genes was compared to known subtilisins and differences were analyzed in more detail by bioinformatic tools. Thus, the proteases are interesting optional candidates for characterization and evaluation for the industrial application. Selected protease genes were cloned and expressed. First results of biochemical investigations will be presented.

## References:

Coker, James A. (2016): Extremophiles and biotechnology: current uses and prospects. In: *F1000Research* 5.

Kalisz, H. M. (1988): Microbial proteinases. In: *Advances in biochemical engineering/biotechnology* 36, S. 1–65.

Maurer, Karl-Heinz (2004): Detergent proteases. In: *Current opinion in biotechnology* 15 (4), S. 330–334.

Siezen, R. J.; Leunissen, J. A. (1997): Subtilases: the superfamily of subtilisin-like serine proteases. In: *Protein science: a publication of the Protein Society* 6 (3), S. 501–523.

## 212-BTP

### Characterization of the marine bone degrading microbiome: a road map for bioinformatics driven enzyme discovery

\*E. Borchert<sup>1</sup>, S. Sánchez-Carrillo<sup>2</sup>, A. García-Moyano<sup>3</sup>, G. E. K. Bjerga<sup>3</sup>, M. Ferrer<sup>2</sup>, U. Hentschel<sup>1</sup>

<sup>1</sup>GEOMAR Helmholtz Centre for Ocean Research Kiel, Marine Symbioses, Kiel, Germany

<sup>2</sup>CSIC - Institute of Catalysis, Applied Biocatalysis, Madrid, Spain

<sup>3</sup>NORCE, Marine Biotechnology, Bergen, Norway

#### Introduction:

Bone is a rather recalcitrant material that remains as leftover by the food industry in the range of millions of metric tons annually. To date only low value chains make use of this potentially valuable bio-composite material. In the ERA-NET Marine Biotechnology project "ProBone" we endeavor to understand the microbial processes in the marine environment that access bone as source for nutrition in otherwise food scarce habitats.

#### Objective:

Understanding and deciphering the mechanisms of marine microbial bone degradation.

#### Methods:

Metagenomic sequencing and analysis, HMM profiling, heterologous expression, amplicon sequencing

#### Results:

Marine microbial metagenomes have been generated from microbial mats and bone-eating *Osedax* worms collected from artificially deployed turkey bones in Byfjorden, outside Bergen, Norway. Differential binning of biofilm and *Osedax* metagenomes yielded 59 high quality bins, spanning 11 different phyla, 14 classes and 23 orders, with 63% of the bins being taxonomically novel. The bins were profiled with HMM models for the occurrence and abundance of 13 different enzyme families, thought to be necessary for bone degradation. More than 800 "bone degrading" enzymes were identified in the reconstructed genomes. The extracted enzyme sequences were investigated via sequence similarity networks and class specific clustering was observed. A portfolio of "bone degrading" enzymes was chosen for heterologous expression in *E. coli* and *B. subtilis*.

#### Conclusion:

Marine microbial bone degradation appears to be a joint effort of different classes of bacteria, with members of the Bacteroidia, Alphaproteobacteria, Campylobacteria and Gammaproteobacteria representing the major players. Especially Bacteroidia bins are enriched in "bone degrading" enzymes, while Alphaproteobacteria are predestined to dissolve bone material via several lactate dehydrogenases and so allowing access to the organic bone compounds for other community members. We present here the predicted metabolic interplay within a specialized microbial community for bone degradation and discuss the implications for potential biotechnological application in food industry.

## 213-BTP

Development of a purely biological, chemical-free phosphorus recovery process in a full-scale wastewater treatment plant for the production of phosphorus-rich organic fertilizer

\*V. Vucic<sup>1</sup>, S. Günther<sup>1</sup>, C. Süring<sup>1</sup>, L. Feissel<sup>1</sup>, F. Schattenberg<sup>1</sup>, J. Lambrecht<sup>1</sup>, Z. Liu<sup>1</sup>, S. Müller<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany

#### Question

Wastewater and wastewater process sludges are no longer considered just as waste but as a raw material source. This is especially the case for phosphorus (P) concentrated within these waste streams. Rising demand for P as non-renewable, critical raw material, especially in agriculture sector calls for P recovery from as many as possible waste streams. Wastewater and connected process streams rich in P are considered a good choice for P recovery. A biological P recovery approach utilizing a novel biological P recovery (BioP-Rec) module for application in a full-scale wastewater treatment plant (WWTP) is designed to produce P rich organic fertilizer. To accomplish the latter, knowledge of the biotic and abiotic parameters that can affect microbial community P uptake, and also influence available reactive P, must be gathered.

#### Methods

P recovery can be carried out at various points of the WWTP. WWTP streams will be characterized with regard to the P content. In addition, different biotic and abiotic bulk parameters will be measured. Flow cytometric microbial community parameters, P content and dependencies between them will be investigated. A flexible BioP-Rec module will be developed and its harvesting capabilities will be investigated together with phosphorus enriched microbial biomass as P rich organic fertilizer.

#### Results

The project aims are to get a complete picture of P balance in a WWTP together with biotic, abiotic and microbial community parameters. Furthermore, to achieve a sludge recovery rate that allows the P content in the biosolids to decline below the limit of 20 g P per kg of dry weight specified by the amended Sewage Sludge Ordinance (1). The BioP-Rec module should produce a biological phosphorus fertilizer that is low in pollutants and is readily plant available.

#### Conclusion

With multidisciplinary scientific approaches of studying microbial communities and P concentration dependencies, we want to overcome the present P resource depletion issue, coupled with environmental protection and new technological development.

#### Literature

1. Verordnung zur Neuordnung der Klärschlammverwertung, Bundesgesetzblatt Jahrgang 2017 Teil I Nr 65 (2017).

## 214-BTP

Production of *N*-methylated amino acids in recombinant

*Corynebacterium glutamicum*

\*A. Kerbs<sup>1</sup>, M. Mindt<sup>1</sup>, V. F. Wendisch<sup>1</sup>

<sup>1</sup>Bielefeld University, Faculty of Biology, Bielefeld, Germany

*N*-methylated amino acids (NMA) are found in many pharmaceutically active compounds and have been shown to improve pharmacokinetic properties as constituents of peptide drugs since *N*-methylation of amino acids typically result in conformational changes, improved proteolytic stability and higher lipophilicity of the peptide drug<sup>1</sup>. NMA are mainly produced chemically or by biocatalysis, however with low yields or high costs for co-factor regeneration. Therefore, a more versatile route for NMA production was established.

*Corynebacterium glutamicum* is used since decades for safe production of amino acids l-glutamate and l-lysine at the million ton scale<sup>2</sup>. Metabolic engineering of *C. glutamicum* enabled the access to other compounds such as amino acids<sup>3</sup> and diamines<sup>4</sup>. Besides, *C. glutamicum* was engineered for production of 2-oxoacids like pyruvate<sup>5</sup>. Implementation of DpkA, an *N*-methylamino acid dehydrogenase from *P. putida*, into *C. glutamicum* strains accumulating 2-oxoacids pyruvate and glyoxylate enabled fermentative production of *N*-methylalanine<sup>6</sup>, sarcosine<sup>7</sup>, and *N*-ethylglycine<sup>8</sup> with alkylamines added to the growth medium. Due to the broad substrate range of DpkA<sup>9</sup>, further *N*-methylated amino acids may be produced using strains providing the respective 2-oxoacid precursor. This work aims at establishing *N*-methylphenylalanine production using DpkA in a phenylpyruvate accumulating *C. glutamicum* strain. Additionally, *N*-methylnorleucine will be produced using DpkA in 2-ketocaproate accumulating *C. glutamicum* strains.

1.Chatterjee *et al.*, *Angew. Chem. Int. Ed.* **52**, 254–269 (2013).

2.Wendisch *et al.*, *Metab. Eng.* (2019). doi:10.1016/j.ymben.2019.03.008

3.Wittmann *et al.*, (Wiley-VCH Verlag GmbH & Co. KGaA, 2017).

4.Wendisch *et al.*, *Appl. Microbiol. Biotechnol.* **102**, 3583–3594 (2018).

5.Wieschalka *et al.*, *Appl. Microbiol. Biotechnol.* **94**, 449–459 (2012).

6.Mindt *et al.*, *Sci. Rep.* **8**, 12895 (2018).

7.Mindt *et al.*, *Bioresour. Technol.* **281**, 135–142 (2019).

8.Mindt *et al.*, *Front. Biotechnol. Bioeng* (2019). doi: 10.3389/fbioe.00232.

9.Muramatsu *et al.*, *Tetrahedron Asymmetry* **15**, 2841–2843 (2004).

## 215-BTP

Acetic acid bacteria strain library screening for best oxidizers of different antibiotic classes

\*M. Bimmer<sup>1</sup>, S. Gruber<sup>1</sup>, W. Liebl<sup>1</sup>, A. Ehrenreich<sup>1</sup>

<sup>1</sup>Technical University Munich, Chair of Microbiology, Freising, Germany

Acetic acid bacteria (AAB) contain numerous membrane-bound dehydrogenases (mDH) with their active site facing to the periplasm that are capable of rapid and incomplete oxidation of a wide range of substrates like mono- and polysaccharides, alcohols and their derivatives in a stereo- and regio- selective manner. Furthermore, it can be observed that AABs are able to withstand several antibiotics. This study investigates the potential of mDHs of AAB to oxidize antibiotics with the result of modification of their structure and efficacy.

The antibiotics cefoxitin, gentamicin, kanamycin A, lincomycin, polymyxin B and tetracycline are tested in final concentrations spanning 5 µM to 100 µM using a whole cell DCPIP assay. This assay uses the change in absorbance maximum generated by electrons from oxidation by mDH transferred via PMS to DCPIP. The resulting reduction of DCPIP can be monitored by decreasing absorption at 530 nm over time. Screening a strain library of AAB identified some *Gluconobacter* strains that show a highly specific oxidation of kanamycin A, lincomycin, polymyxin B in low concentrations of 5 or 10 µM and above, as well as oxidation of cefoxitin and tetracycline in higher concentrations of 75 µM and above.

These results will be used to identify the responsible mDHs and characterize the generated oxidation products in further experiments. While AAB are no pathogenic organisms mDHs can also be found in several Pseudomonads and related organisms among which are numerous pathogenic strains. The role of mDHs for intrinsic resistance of such organisms against antibiotics is therefore an interesting aspect of this work.

## 216-BTP

Biotransformation of terpenes: seek for new sustainable materials

\*A. L. Hiller<sup>1</sup>, M. Böckers<sup>1</sup>, L. Antelo<sup>2</sup>, S. R. Waldvogel<sup>3</sup>, E. Thines<sup>1,2</sup>

<sup>1</sup>Johannes Gutenberg-University, Institute of Molecular Physiology, Mainz, Germany

<sup>2</sup>Institute of Biotechnology and Drug Research gGmbH, Kaiserslautern, Germany

<sup>3</sup>Johannes Gutenberg-University, Institute of Organic Chemistry, Mainz, Germany

The search for new sustainable materials was significantly focused in recent years. Due to the scarcity of traditional petroleum derivatives, these materials must be produced independently of oil derivatives and without a negative impact on the CO<sub>2</sub> balance. Moreover, they have to be biodegradable so that they do not subsist indefinitely in the environment, as it is the case with plastics today. If these new materials are synthesized from natural compounds and through a biological process, they will be easier to be processed by nature directly.

There are more than 25 000 known terpenes and terpenoids, being the largest group of natural products. They also are present in industrial waste products like turpentine oil, a byproduct in the pulp and paper industry, therefore are inexpensive and available in large quantities.

In our group, we made a pre-selection of fungi from the collection of the IBWF (containing more than 20.000 strains) which are able to tolerate diverse terpenes and metabolize them. Several filamentous fungi are able to produce mono- and diols as well as mono- and diepoxide derivatives of the terpene substrates.

These, and other substances, may be used as monomers for the production of new materials from renewable sources. Therefore, we will focus on interesting substances that may be used as building blocks in chemical synthesis.

---

## 217-BTP

New metabolites from old pathways - exploring the potential of artificial biosynthetic pathways to produce novel compounds

\*J. E. Kufs<sup>1,2</sup>, S. Hoefgen<sup>1</sup>, J. Rautschek<sup>1</sup>, V. Valiante<sup>1,2</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Biobricks of Microbial Natural Product Syntheses, Jena, Germany

<sup>2</sup>Friedrich Schiller University, Jena, Germany

Natural products are a valuable source for novel drug candidates with beneficial effects for the society regarding pharmaceutical, agricultural and environmental applications. However, most natural products are not easily accessible by cultivation of their natural hosts due to slow growth rates or minor production yield of the desired compound. Furthermore, the synthetic production of complex natural products is often associated with a high effort regarding chemical syntheses.

In order to find new technologies for the discovery of novel compounds, we developed a system for the combinatorial biosynthesis of plant polyketide derivatives in *E. coli* using a multi-gene single-plasmid expression vector. First, a coumarate-CoA ligase from *Nicotiana tabacum*, as well as a chalcone synthase and chalcone isomerase from *Arabidopsis thaliana* were heterologously expressed in *E. coli* and analyzed for polyketide production. HPLC and HR-MS data showed the *in vivo* production of the flavanone, naringenin. We also included further tailoring enzymes that act on the flavanone to produce naringenin derivatives of high structural diversity. Additionally, we determined the promiscuity of the chalcone synthase to accept various coumaroyl-CoA derivatives to further modify the core polyketide. In this study, we demonstrated the flexibility and applicability of our bacterial multi-gene expression system to produce natural and artificial derivatives of an already known compound.

---

## 218-BTP

Synthesizing diverse polyketide building blocks from carboxylic acids via a one-pot enzymatic route

\*A. Bennion<sup>1</sup>, P. D. Gerlinger<sup>1</sup>, T. J. Erb<sup>1</sup>

<sup>1</sup>Max Planck Institute for Terrestrial Microbiology, Biochemistry and Synthetic Biology, Marburg, Germany

Polyketides represent a large group of compounds displaying a myriad of valuable biological activities including antibacterial, antifungal and immunosuppressant. Polyketides are synthesized by enzyme complexes called polyketide synthases (PKSs) via the stepwise condensation of alkylmalonyl-CoA extender units after priming with an acyl-CoA starter unit. Though PKSs show tolerance toward non-natural extender units, the structural and functional diversity of downstream products is limited by the lack of convenient synthesis routes for extender units with chemically active rest groups. To address this issue, we aim to develop a fully enzymatic route for converting carboxylic acids into atypical acyl-CoA starter units and their respective extender units through subsequent oxidation and carboxylation. We have selected a representative set of three CoA ligases from different clades, each described to possess a unique substrate specificity. We screened each enzyme with a range of substrates including phenylated, halogenated, and

aminated carboxylic acids. We first used LCMS to observe the formation of product over time at varying physiological conditions and then used to spectrophotometry to determine their Michaelis-Menten kinetics. The most promising CoA ligase was then coupled with an acyl-CoA oxidase and enoyl-CoA carboxylase/reductase, demonstrating the ability to enzymatically generate a library of atypical and chemically diverse extender units. Furthermore, this initial screening identified potential candidates for enzyme engineering and pointed toward a phylogenetic clade of CoA ligases from which we will search for homologs with improved substrate promiscuity and reaction velocity. This convenient, one-pot synthesis route can be used to provide a versatile set of middle- and long-chain starter and extender units with a variety of rest groups in order to introduce structural and functional diversity in the downstream products of PKSs to produce novel compounds.

---

## 219-BTP

Self-immobilizing biocatalysts for continuous stereoselective reactions in microfluidic systems

\*P. Bitterwolf<sup>1</sup>, K. S. Rabe<sup>1</sup>, C. M. Niemeyer<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Institute for Biological Interfaces 1, Karlsruhe, Germany

Biocatalytic flow processes enable the sustainable and continuous production of value-added chemicals for the industrial "white" biotechnology sector.<sup>1,2</sup> Apart from optimizing the biocatalytic performance by methods of directed protein evolution, maximizing the effective immobilization capabilities of enzymes is essential to enable efficient fluidic micro reactor processes. In order to retain the biocatalysts under continuous flow conditions, we are developing self-immobilizing biocatalysts, such as whole-cells<sup>3</sup> and isolated enzymes<sup>4</sup> for functionalization of superparamagnetic micro particles, as well as reactor modules manufactured by 3D printing of bioinks containing thermostable enzymes<sup>5</sup> To further increase the volumetric activity of such reactors, we have recently developed self-assembling all-enzyme hydrogels with cofactor-regenerating capabilities, consisting of either an (*R*)- or an (*S*)-enantiospecific ketoreductase combined with the NADPH-regenerating glucose 1-dehydrogenase.<sup>6,7,8</sup> Mounted in microfluidic reactors, the gels show excellent stereoselectivity (> 99% ee.) with near quantitative conversion (> 90%) for the reduction of prochiral ketones along with high robustness under process and storage conditions. The gels constitute a compartment in which reaction intermediates are retained, thereby enabling extraordinarily high total turnover numbers of the expensive cofactor NADP(H). Coupling of such reactor modules allows to facilitate multi step reaction cascades.

## References

- [1] R. A. Sheldon, J. M. Woodley, *Chem. Rev.* **2017**.
- [2] K. S. Rabe, J. Muller, M. Skoupi, C. M. Niemeyer, *Angew. Chem. Int. Edit.* **2017**.
- [3] T. Peschke, K. S. Rabe, C. M. Niemeyer, *Angew. Chem. Int. Edit.* **2017**.
- [4] T. Peschke, M. Skoupi, T. Burgahn, S. Gallus, I. Ahmed, K. S. Rabe, C. M. Niemeyer, *ACS Catal.* **2017**.
- [5] M. Maier, C. P. Radtke, J. Hubbuch, C. M. Niemeyer, K. S. Rabe, *Angew. Chem. Int. Edit.* **2018**.

[6] T. Peschke, P. Bitterwolf, S. Gallus, Y. Hu, C. Oelschlaeger, N. Willenbacher, K. S. Rabe, C. M. Niemeyer, *Angew. Chem. Int. Edit.* **2018**.

[7] T. Peschke, P. Bitterwolf, S. Hansen, J. Gasmi, K. S. Rabe, C. M. Niemeyer, *Catalysts* **2019**.

[8] P. Bitterwolf, S. Gallus, T. Peschke, E. Mittmann, C. Oelschlaeger, N. Willenbacher, K. S. Rabe, C. M. Niemeyer, *Chem. Sci.* **2019**

---

## 220-BTP

Identification and heterologous expression of putative NRPS-like and PKS coding genes from *Guignardia bidwellii* in

### *Magnaporthe oryzae*

\*H. Neumann<sup>1</sup>, A. Borger<sup>1</sup>, L. Schirling<sup>1</sup>, L. Antelo<sup>2</sup>, K. Andresen<sup>1</sup>, E. Thines<sup>1,2</sup>

<sup>1</sup>Johannes Gutenberg-University Mainz - Institute of Molecular Physiology, Microbiology and Wine Research, Mainz, Germany

<sup>2</sup>Institute of Biotechnology and Drug Research (IBWF), Kaiserslautern, Germany

*Guignardia bidwellii* is the causal phytopathogenic fungus of black rot in vines posing a massive threat to organic viticulture. The phytotoxic dioxolanones phenguignardic acid and guignardic acid have been identified as potential virulence factors involved in the infection process of the black rot fungus.

To date a genetic manipulation of *G. bidwellii* has not been successfully established and therefore a heterologous expression system has been established for the study of the secondary metabolites involved in the biotic interaction.

The genome of *G. bidwellii* has been sequenced and two putative NRPS-like coding genes have been identified. Moreover, transcriptome analysis led to identification of one polyketide-synthase gene which expression correlates with the production of dioxolanones.

In contrast to NRPS, NRPS-likes consist of only one module with adenylation, thiolation and thioesterase domains, but lacking a condensation domain. The catalyzed reaction is the coupling of exactly two deaminated aromatic amino acids as building blocks, which can be modified by other proteins.

These three genes (GbNRPS-like1/2 and GbPKS1) have been introduced into *Magnaporthe oryzae* in order to express these enzymes under the control of a constitutive promoter.

Several new compounds could be isolated from the culture of the heterologue expression strains that are not present in the wild type of *M. oryzae*: phenguignardic acid, when GbNRPS-like1 was expressed, and guignardianone C, by the co-expression of the GbNRPS-like1 and the GbPKS1 genes.

---

## 221-BTP

### Disruption of the PEP-Pyr-OAA node as a strategy to improve ectoine production in *Halomonas elongata*

\*K. Hobmeier<sup>1</sup>, K. Pflüger-Grau<sup>1</sup>, A. Kremling<sup>1</sup>, A. Marin-Sanguino<sup>1</sup>

<sup>1</sup>Technical University of Munich (TUM), Systems Biotechnology, Garching, Germany

The compatible solute ectoine is a natural stress-protection molecule highly demanded in cosmetics and biomedical industries. It is naturally produced by many organisms including *Halomonas elongata* which can survive at salt

concentrations up to 30% by accumulating ectoine in its cytoplasm. To develop an improved ectoine producing strain, the leaky mutant was chosen as a starting point for additional modifications [Grammann et al., 2002]. By combining experiments and computational simulations we identified areas in the central metabolism crucial for ectoine synthesis, like the PEP-Pyr-OAA node. This node includes the anaplerotic reactions that replenish the OAA drawn out of the TCA cycle. Within the node two gluconeogenic enzymes PEPCK and NADP-ME were selected for knock-out (KO) by homologous recombination. A screening of the KO mutants (*H. elongata*  $\Delta pckA$  and  $\Delta pckA \Delta maeB$ ) in a 96-well plate testing various salt concentrations and carbon sources (glycolytic and gluconeogenic) revealed that inhibitory effects on the growth are present only at low salt concentrations regardless of the carbon source. In a shaking flask experiment on glucose at 1 M salt the growth rates of both mutants were observed to be 10 to 20% slower. However, they achieved ectoine titers of 40% higher than the leaky strain. The disruption of the PEP-Pyr-OAA node by *pckA* and *maeB* KO initially leads to an increased supply of OAA due to the suppression of futile cycles. In non-halophilic bacteria such as *E. coli*, an increase in OAA reduces growth rates by decreasing anaplerotic flux through Ppc. In contrast, *H. elongata* was shown to behave differently, presumably due to an alternative anaplerotic reaction catalyzed by a salt-dependent membrane bound Oxaloacetate decarboxylase (*oad*). In conclusion, the PEP-Pyr-OAA node was identified as an important junction for metabolic engineering towards more powerful ectoine producing strains.

Grammann Katrin, et al.: "New type of osmoregulated solute transporter identified in halophilic members of the bacteria domain: TRAP transporter TeaABC mediates uptake of ectoine and hydroxyectoine in *Halomonas elongata* DSM 2581T." *J Bacteriol.* 2002 Jun;184(11):3078-85.

---

## 222-BTP

Development of a two-step hydrocyclone system for continuous processing in biotechnological production

\*H. Al-Kaidy<sup>1</sup>, J. Millauer<sup>2</sup>, B. U. Wilhelm<sup>2</sup>, J. Bader<sup>1</sup>

<sup>1</sup>Beuth University of Applied Sciences, Life Sciences and Technology, Berlin, Germany

<sup>2</sup>bbi-biotech GmbH, Berlin, Germany

Hydrocyclones are widely used in all areas of particle separation [1]. However, commercially available systems have not been used for cell harvesting since cells are sensitive to shear forces and the separation of cell debris is another major challenge due to short retention times and prevailing g-forces. In order to use hydrocyclones for cell culture separation, a special control of the technological parameters such as flow velocity, inflow rate and g-forces is required.

In the present project, a two-stage, fully automated continuous separation process is being developed. In the first step of the separation, the cells will be separated from the liquid phase in a hydrocyclone and will then be returned to the fermenter. In a second step, the solution is led through another hydrocyclone where cell fragments and released DNA will be separated. This can be achieved by an automatized precipitation process, which will be developed in this project. Finally, the clear supernatant will be further processed by a continuous chromatography system.

Using CAD and 3-D printing, prototypes of hydrocyclones in different geometries and radii were developed. In these, the cell separation of different cells is model-tested with yeasts and animal cells and characterized with respect to the cell

separation behavior in the hydrocyclone. Flow rates will be correlated with separation rates, the process stability will be determined with respect to different process parameters.

In first tests, it could be shown that the use of hydrocyclones is basically suitable for the separation of yeast cells. The first prototypes showed also that at flow rates of 500 ml min<sup>-1</sup>, the viability of the CHO cells after separation is about 77 percent. In order to optimize the separation process, there is an ongoing research to find the optimal design of the hydrocyclone, the best precipitation medium and suitable process parameters.

[1] Long Ni et al. (2019): "Optimizing Geometric Parameters in Hydrocyclones for Enhanced Separations: A Review and Perspective", Separation & Purification Reviews, DOI:10.1080/15422119.2017.1421558

## 223-BTP

Employing microfluidic bioreactors for machine-assisted cultivation and analysis of productive biofilms

\*P. Lemke<sup>1</sup>, K. S. Rabe<sup>1</sup>, H. Horn<sup>2</sup>, J. Gescher<sup>3</sup>, C. M. Niemeyer<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology, Institute for Biological Interfaces, Karlsruhe, Germany

<sup>2</sup>Karlsruhe Institute of Technology, Engler-Bunte-Institut, Water Chemistry and Water Technology, Karlsruhe, Germany

<sup>3</sup>Karlsruhe Institute of Technology, Institute for Applied Biosciences, Karlsruhe, Germany

Biofilms are one of most abundant forms of life in the ecosystem, however they are far from being fully understood. The self-assembled network of diverse bacterial species and other microbial organisms forms a complex community, which is usually robust and stress resistant. On the one hand these properties make them hard to handle and unwanted in the field of medicine, on the other hand they can potentially be utilized in terms of biotechnology or environmental biology. Employing biofilms could have great impact for the production of pharmaceuticals or the selective removal of undesirable substances. However, a much more detailed analysis and better biological understanding of biofilm genesis, growth and productivity is the key to successful application of these complex communities.

By utilizing microfluidic bioreactors for the cultivation of productive biofilms a precise control of the conditions and biomass sampling can be achieved. This, however, also requires an analytical method compatible with the reactor. To this end a dedicated platform for automated cultivation and analysis of multi-species biofilm under flow has been developed[1]. The platform is designed to support multiple microfluidic flow cells of various geometries while keeping environmental conditions such as temperature and flow rate constant. Flow cells can be easily manufactured as PDMS chips or PDMS-glass hybrids[2], are compatible with analytical instrumentation such as automated light microscope, fluorescence reader or optical coherence tomography devices. The advantages of automated sampling are the ability to only remove very small amounts of sample volume while minimally invading the grown biofilm as well as in high spatiotemporal resolution in analysis of metabolites and biofilm composition. These features enable the detailed investigations of biocatalytically active biofilms in order to understand and optimize such biological systems.

## References

- [1] S. H. Hansen et al. "Machine-assisted cultivation and analysis of biofilms" Sci. Rep. **2019**.  
[2] M. Grösche et al. "Microfluidic Chips for Life Sciences—A

Comparison of Low Entry Manufacturing Technologies" Small **2019**.

## 224-BTP

Steryl esters, a new class of storage compounds in steroid-degrading bacteria

\*J. Holert<sup>1,2</sup>, K. Brown<sup>2</sup>, A. Hashimi<sup>2</sup>, B. Philipp<sup>1</sup>, L. Eltis<sup>2</sup>, W. Mohn<sup>2</sup>

<sup>1</sup>Westfälische Wilhelms-University Münster, Münster, Germany

<sup>2</sup>University of British Columbia, Department of Microbiology & Immunology, Vancouver, Canada

Steryl esters are important neutral-lipid storage compounds in eukaryotes, but their formation has not been reported in bacteria. Strikingly, sterols can be used as growth substrates by many oleaginous bacteria, particularly by members of the Corynebacterineae order, which are known to produce other high-value neutral lipids such as triacylglycerols (TAG) and wax esters (WE). Here we demonstrate that selected *Rhodococcus*, *Mycobacterium*, and *Mycolatopsis* strains as well as some strains of the oligotrophic marine Gammaproteobacteria (OMG) group are also able to synthesize steryl esters and to sequester them into cytoplasmic lipid droplets when grown on sterols.

Using GC-MS based neutral lipid analysis tools and lipid droplet staining techniques, we found that cholesteryl esters maximally accumulated under nitrogen-limiting conditions, suggesting that steryl ester formation plays a crucial role for storing excess organic electron donors and carbon under adverse conditions. *Rhodococcus jostii* RHA1, an emerging model organism for the biotechnological production of valuable TAG and WE storage compounds, was able to synthesize phytosterol- and cholesteryl esters, the latter reaching up to 7% of its cellular dry weight and 70% of the lipid droplets. Purified lipid droplets from cholesterol-grown RHA1 contained cholesteryl esters, free cholesterol and TAGs. Interestingly, the majority of fatty acids stored in TAGs and cholesteryl esters were odd-numbered. In addition, we found formation of cholesteryl esters in *Mycobacterium tuberculosis* when grown with cholesterol plus an additional fatty acid substrate.

This study provides the first evidence for steryl ester accumulation in free-living and pathogenic bacteria and offers novel approaches for the biotechnological production of steryl esters with potential applications in the functional food, nutraceutical, and cosmetic industries. As such, phytosterols and their esters were recognized as potential agents to lower serum cholesterol levels and are approved in many countries as safe food additives for the use in functional foods.

Holert et al., 2019, AEM.02353-19; DOI: 10.1128/AEM.02353-19

## 225-BTP

A NanoBiT Metabolite Sensor based on the *Synechocystis* sp. PCC6803 PII Signaling Protein with PII-interacting protein X

\*R. Rozbeh<sup>1,2</sup>, K. Forchhammer<sup>1</sup>

<sup>1</sup>University of Tuebingen, organismic interactions, Tübingen, Germany

<sup>2</sup>„ Germany

Proteins are essential components for the main physiological process of living cells by making interactive networks that are responsive to evolving conditions. The interactions are affected by a complex interplay of structural conformations

and relationships with effector molecules. In order to analyze these interactions in vivo, split luciferases proteins are applied because of their sensitivity and simplicity. Recently a new method called NanoLuc Binary Technology (NanoBiT) has been developed based on the smallest luciferase enzyme termed NanoLuc. The NanoLuc luciferase is split in two subunits, a Large Bit (18 kDa polypeptide) and Small Bit (1.3 kDa peptide), which only weakly associate, so that their assembly, which restores luciferase activity, depends on the interaction characteristics of target proteins onto which they are fused. We used this technology to develop a new NanoBiT sensor based on the interaction of signal transduction protein PII from *Synechocystis* sp. PCC6803 with the PII-interacting protein X (PipX). Experimental result displayed that the complex of PII and PipX proteins shows luminescence when Large Bit is attached at the C-terminus of PII protein and Small Bit is attached at the C-terminus of PipX protein with appropriate linker sequences. Further studies confirmed that the complex responds to the presence of ATP, ADP and ATP/ 2-oxoglutarate in vitro. In accord with previous surface plasmon resonance data, the affinity of complex is higher in the presence of ADP compared to ATP, while ATP/2-oxoglutarate can dissociate the complex completely. Therefore, the PII-PipX NanoBiT sensor is functional in vitro to study metabolite effects and can now be used for in vivo applications.

## 226-BTP

### 3D-Printed Flow Reactor Modules for Chemoenzymatic Synthesis

\*M. Peng<sup>1</sup>, K. S. Rabe<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technologie (KIT), Institute for Biological Interfaces (IBG-1) - Biomolecular Micro- and Nanostructures, Eggenstein-Leopoldshafen, Germany

The compartmentalization of chemical reactions is a basic principle in nature, which can be implemented in technical processes by performing reaction cascades with physically separated enzymes. For the development of novel approaches in biocatalysis this principle is a major source for innovations and is therefore mimicked in several ways. The immobilization of biocatalysts in a fluidic setup is one way to achieve compartmentalization and thus precise control over artificial reaction cascades. Established state-of-the-art technologies to arrange enzymes for sequential reactions require chemical or genetically encoded modifications of the target enzymes, which can negatively influence the activities and specificities of the immobilized enzymes. We recently demonstrated that unmodified thermostable enzymes can be immobilized by encapsulation in a simple and inexpensive, agarose-based thermoreversible hydrogel.<sup>1,2</sup> The method allows the on-demand and on-site production of catalytically active reactor modules with various shapes based on grid-structured circles and polygons using a commercially available easy-to-operate 3D printing process. As exemplary biocatalysts, we used an esterase and an alcohol dehydrogenase from thermophilic organisms as well as a phenacrylate decarboxylases and a ketoisovalerate decarboxylase from mesophilic organisms. The latter was thermostabilized by rational protein engineering combined with directed protein evolution. We showed that the resulting 3D-printed modules can be utilized for continuous, multi-step sequential biotransformations, which can also be run in parallel fluidic setups. Via simply changing the number of stacked modules, the reaction profile could be tuned. We further expanded the scope of this approach by integrating phenacrylate decarboxylases reactor modules into a chemoenzymatic workflow.<sup>2</sup>

1 M. Maier, C. P. Radtke, J. Hubbuch, C. M. Niemeyer, K. S. Rabe (2018), Angew. Chem., Int. Ed. 19: 5539–5543

2 M. Peng, E. Mittmann, L. Wenger, J. Hubbuch, M. K. M. Engqvist, C. M. Niemeyer, K. S. Rabe (2019), Chem. - Eur. J., doi: 10.1002/chem.201904206

## 227-BTP

Enrichment of microbial communities from midgut and hindgut of Sun beetle larvae (*Pachnoda marginata*)

\*B. Grosch Schroeder<sup>1</sup>, S. Kleinstaub<sup>1</sup>, W. Logrono<sup>1</sup>, M. Nikolausz<sup>1</sup>  
<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology (UMB), Leipzig, Germany

Certain beetle larvae have complex gut microbiota capable of effective conversion of lignocellulosic biomass, therefore, this study aimed to enrich effective cellulose degrading microorganisms from the microbiota of the larva of *Pachnoda marginata*. Two enrichment strategies from midgut and hindgut of the *Pachnoda marginata* beetle larva were compared. In the strategy 1, the inoculum was prepared partially under oxic conditions and in the strategy 2 the inoculum was prepared under anoxic conditions with a larger inoculum size. The results presented correspond to three transfers, always carried out at 30 days of cultivation. The microbiota was enriched in a modified DSMZ 1036 medium with wheat straw as substrate, at pH 9 and incubated at 37 °C. For each experiment, samples of microbial biomass, liquid and gas phase were collected every 5 days. The amplicon sequencing of *mcrA* gene showed that the methanogens enriched in strategy 1 were significantly different from ones in strategy 2. In the strategy 1 there was a predominance of *Methanobrevibacter* genus for midgut and hindgut enrichment cultures whereas in the strategy 2 *Methanococcus* was also abundant beside *Methanosarcina*. The physiological parameters have shown that the efficiency in methane production were higher for strategy 2, while the highest acetic acid concentrations were observed for the strategy 1. These results suggest that the volatile fatty acids (VFAs) in the strategy 1 have not completely been converted to methane. On the other hand, the strategy 2 showed a highest efficiency in the conversion of VFAs to methane. It could be explained by the effect of inoculation strategy on the microbial community composition in the strategy 2. As the gut enrichment cultures from strategy 2 were able to produce acetic acid and the community included aceticlastic methanogens, the activity of aceticlastic methanogens could explain the lack of accumulation of acetic acids. Based on the presented results we could infer that the inoculum preparation play an important role in the enrichment cultures by influencing the microbial community.

## 228-BTP

Screening of Signal Peptides for Secretion of Heterologous Proteins in *C. glutamicum* using split GFP

\*C. Müller<sup>1</sup>, I. Chika Linda<sup>1</sup>, V. Waffenschmidt<sup>1</sup>, W. Wiechert<sup>1</sup>, M. Oldiges<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, IBG-1: Biotechnology, Jülich, Germany

*Corynebacterium glutamicum* is an important industrial platform organism for the production of a broad set of metabolite products and small molecules, such as amino and organic acids. Moreover, the Gram-positive *C. glutamicum* is able to secrete proteins via SEC pathway sufficiently.

A huge variety of signal peptides (SP) for protein secretion is known. However, it is still a challenge to predict a suitable SP for high-level secretion of a specific heterologous target protein. Instead, SP libraries must be tested for each host and target protein. This requires the construction of a suitable strain library for every SP target protein combination



and the determination of secretion performance under bioprocess-relevant conditions.

The objective is to establish a high throughput screening system for heterologous protein secretion in *C. glutamicum*. Therefore, a library of approx. 120 different *Bacillus subtilis* SP for SEC secretion of heterologous proteins is constructed semi-automatically. To investigate the influence of translation initiation, the distance between Shine-Dalgarno sequence and start codon is varied. The well-known model protein cutinase from *Fusarium solani pisi* is used. Individual strain performance is tested on a lab automation platform enabling miniaturized and parallelized cultivation experiments. The quantity of secreted proteins is determined by split GFP assay. By this established detection method, target protein concentration in culture supernatant is measured activity-independently. This is enabled by attachment of the 11th  $\beta$ -sheet of GFP (GFP11) to the target protein and addition of the non-fluorescent GFP1-10 protein as detector reagent. The fluorescence of the self-associated GFP fragments provides an estimation about cutinase secretion performance.

It could be successfully shown that the split GFP assay is suitable for monitoring secretion performance of a heterologous cutinase in *C. glutamicum*. The complete workflow from cultivation to detection of secreted proteins is about to be done fully automated to provide sufficient throughput for SP strain library screening.

---

### 229-BTP

Development of automated workflows for the accelerated production and characterization of catalytically active inclusion bodies

\*K. Küsters<sup>1</sup>, S. Albert<sup>1</sup>, W. Wiechert<sup>1</sup>, M. Oldiges<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, IBG-1, Jülich, Germany

Enzymes as biocatalysts play an important role in industrial biotechnology for decades. Beside the direct application of purified soluble enzyme, many immobilization techniques are known to generate immobilized enzyme variants. This is often accompanied by reduction of overall enzyme activity, but also stabilizing effects are well known. During intracellular overexpression of enzymes in *Escherichia coli* in combination with a linker and an aggregation tag catalytically active inclusion bodies (CatIBs) were found. CatIBs are known for their high stability as well as their easy and low cost production and can provide an interesting alternative approach to immobilization of enzymes. However, since their discovery a few years ago, only a few CatIB variants were described in literature. Although a substantial number of specific aggregation tags and linker sequences are known, there is not enough structural understanding to predict a suitable combination of aggregation tag and linkers to successfully generate a CatIB for a target protein. One reason is the required time and resources to establish manual cloning and characterization workflows of different target genes in combination with many linker and aggregation tags.

The aim of the project is to automate the molecular biology construction workflows as well as the characterization processes to accelerate the generation of successful CatIB variants. As a prototypic example the lysine decarboxylase (cadA) from *E. coli* is used as model for CatIB formation.

Therefore, lysine decarboxylase gene was combined with ten linker/aggregation tag variations and assembled via Golden Gate cloning in an expression plasmid. Strikingly, the ten variants showed different levels of CatIB formation based on

microscopic observation, proving the hypothesis that linker/aggregation tag combinations clearly influence the ability to build CatIB for lysine decarboxylase. Afterwards, the enzymatic activity of the inclusion bodies was tested via activity measurement in microplate format and also showed different residual CatIB activities of the variants. The developed automated workflows enable a fast detection of successful CatIB variants.

---

### 230-BTP

Efficient in-line extraction system for selective 1-hexanol production from synthesis gas fermentation

\*P. Kottenhahn<sup>1</sup>, G. Philipps<sup>1</sup>, S. Jennewein<sup>1</sup>

<sup>1</sup>Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Department for Industrial Biotechnology, Fraunhofer IME, Aachen, Germany

In recent years, *Clostridium carboxidivorans* P7 has moved into focus of scientific interest due to its rare ability to metabolize Syngas, a mixture of H<sub>2</sub>, CO, CO<sub>2</sub> (and N<sub>2</sub>) directly to the industrially relevant compound hexanol. This medium length alcohol is used in a wide range of applications as a solvent and can be further processed into surfactants, plasticizers, plastics and insect repellent. Furthermore, the use of syngas fermentation products such as butanol and hexanol has been discussed as a potential sustainable drop in fuel.

Other fermentation products of *C. carboxidivorans* P7 are ethanol and butanol and the corresponding acids, namely acetate, butyrate and caproate. Product titers and ratios are highly dependent on gas and medium composition and fermentation parameters. Hexanol titers produced by *C. carboxidivorans* P7 were low until recent publications concerning fermentation design reported optimized fermentation parameters and media composition for efficient alcohol production. To identify a potential limiting factor in hexanol biosynthesis with *C. carboxidivorans* P7, we determined inhibitory concentrations of the product. The inhibitory hexanol concentration we found matched the highest hexanol titers produced in other studies. This hints at product inhibition being the currently limiting factor in hexanol production. To circumvent product toxicity in syngas fermentation, we developed an in-line extraction system for hexanol. A threefold increase of product concentration was found when oleyl alcohol was used. Hexanol titers above toxic concentrations were reached with the product being highly concentrated in the extracting agent with only low titers of other products. This opens up the possibility to further improve hexanol yields beyond theoretically toxic titers, while simultaneously allowing for easy product harvest and purification from the extracting agent.

---

### 231-VPP

Parallel and scalable workflow for the identification and analysis of Phages in sequencing data

\*M. Marquet<sup>1</sup>, M. W. Pletz<sup>1</sup>, O. Makarewicz<sup>1</sup>, A. Viehweger<sup>2</sup>, M. Hoelzer<sup>3</sup>, C. Brandt<sup>1</sup>

<sup>1</sup>University Hospital Jena, Institut für Infektionsmedizin und Krankenhaushygiene, Jena, Germany

<sup>2</sup>University Hospital Leipzig, Institute of Medical Microbiology, Leipzig, Germany

<sup>3</sup>EMBL-EBI European Bioinformatics, Wellcome Trust Genome Campus, Hinxton, United Kingdom

Phages will be increasingly used as platforms for antigen display, in pathogen detection, or as vaccines, e.g., as an alternative for the treatment of multiresistant bacteria. Long

read sequencing technologies, such as nanopore sequencing, allows for complete phage-genomes sequencing anytime, with comparatively low investment. Sequencing data, in general, allows us to study the occurrence, spread and the type of bacteriophages, which in return increases the demand for automated pipelines. Numerous phage prediction tools have been introduced. They all differ in dependencies, their language, the usability, the output data and more, massively increasing the difficulties for a broader audience to use.

## Methods

The tool "What-the-Phage" (WtP) is written in Nextflow and utilizes Docker containers for a simplistic workflow execution in any Linux environment. All containers are written, tested and stored on [hub.docker.com/u/multifractal](https://hub.docker.com/u/multifractal). WtP automatically utilizes these containers which in return means that the Installation of specific tools is no longer needed. All dependencies or databases are automatically downloaded. WtP is freely available at [github.com/replikation/What\\_the\\_Phage](https://github.com/replikation/What_the_Phage), which includes a simple installation routine for Nextflow/Docker.

## Results

We established a reproducible, scalable and easy-to-use workflow for phage identification and analysis. Our tool combines currently six established phage identification tools: Virfinder, PPR-Meta, Virsorter, Deepvirfinder, Metaphinder, and MARVEL. WtP analyses and summarizes the results gathered from sequencing data or direct nanopore raw reads. For this, each sample is combined into heatmaps for comfortable results interpretation. Moreover, multiple samples are computed in parallel and if less hardware is available, WtP decreases the parallelization automatically.

## Conclusion

Our ongoing effort is to incorporate additional identification tools, more downstream analysis, and visual phage annotation. The modular setup allows us to add additional tools easily. WtP is a highly robust and stable pipeline for the identification and analysis of Phages which can easily handle both single and multi-sample inputs.

## 232-VPP

Spatiotemporal binding dynamics of the Lsr2-type xenogeneic silencer CgpS during prophage induction in *Corynebacterium glutamicum*

\*M. Hünnefeld<sup>1</sup>, A. Filipchyk<sup>1</sup>, J. Frunzke<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, IBG-1: Biotechnologie, Jülich, Germany

### 1. Introduction

Virus-derived DNA represents a predominant cause for strain-specific differences within a bacterial species. Due to potentially toxic gene products, the expression of viral genes requires a stringent regulation. In a recent study, we have characterized the function of the Lsr2-type protein CgpS encoded by the CGP3 prophage of the actinobacterium *Corynebacterium glutamicum*<sup>1</sup>. Genome-wide analysis of DNA-binding revealed that CgpS binds to AT-rich genomic regions and confirmed the CGP3 prophage as the predominant target of CgpS. Interference with CgpS binding resulted in the induction of the CGP3 prophage element, highlighting the essential role of CgpS for maintaining the lysogenic state of the CGP3 prophage<sup>2</sup>.

## 2. Objectives

This study focused on the time-resolved analysis of the DNA binding dynamics of CgpS in the course of prophage induction in order to decipher its role on the control of the phage life cycle.

## 3. Methods

Time-resolved chromatin affinity purification and sequencing (ChAP-Seq) analysis combined with proteome and transcriptome analyses.

## 4. Results

Our data revealed that CgpS shows a dynamic binding behavior under inducing conditions. However, CgpS did not fully dissociate from the CGP3 region during induction, but rather emphasized a remodeling of the silencer nucleoprotein complex at the prophage region and a partial reallocation to further targets in the host genome. Among these host targets were several genes encoding global transcriptional regulators, proteins involved in cell envelope biosynthesis, and proteins involved in DNA replication and repair.

## 5. Conclusion

Altogether, our studies provide comprehensive insights into genome dynamics during the process of prophage induction and highlight the important role of Lsr2-type proteins in the coordination of the phage life cycle.

1. Pfeifer, E., Hünnefeld, M. *et al.* Silencing of cryptic prophages in *Corynebacterium glutamicum*. *Nucleic Acids Res.* 44, 10117–10131 (2016). doi:10.1093/nar/gkw692
2. Pfeifer, E., Hünnefeld, M., Popa, O. & Frunzke, J. *Impact of Xenogeneic Silencing on Phage-Host Interactions*. *Journal of Molecular Biology* (Academic Press, 2019). doi:10.1016/j.jmb.2019.02.011

## 233-VPP

**Investigation of Ionic Liquids for concentration of viral particles of aqueous samples**

\*J. Sommer<sup>1</sup>, B. Bromberger<sup>2</sup>, T. Gundolf<sup>1</sup>, R. Kalb<sup>3</sup>, P. Rossmann<sup>1,2</sup>, P. J. Mester<sup>1</sup>

<sup>1</sup>Institute of Food Safety, Food Technology and Veterinary Public Health Department for Farm Animals and Veterinary Public Health, Christian Doppler-Laboratory for Monitoring of Microbial Contaminants, Vienna, Austria

<sup>2</sup>Institute of Food Safety, Food Technology and Veterinary Public Health Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Unit of Food Microbiology, Vienna, Austria

<sup>3</sup>Proionic Production of Ionic Substances GmbH, Grambach, Austria

**Introduction:** Due to ongoing globalization, an increased number of virus disease outbreaks has been recorded. Although recorded outbreaks are increasing, detection methods are not underlying this trend. This results in a high number of not verified virus-associated outbreaks, which mainly can be related to determination limits of currently used detection methods. Moreover, detection of viral particles out of native, aqueous environmental samples is not very promising, since preceding separation methods, like ultracentrifugation or various membrane-filter techniques, are limited.

**Objectives:** Therefore, the aim of this study was the development of a two-phase-system, consisting of an Ionic Liquid-phase (IL-phase), for concentration of different viruses.

**Material & methods:** Fourteen different hydrophobic ILs were screened for their phase-concentration potential of viral particles. The viruses were selected according to their capsid and genome structure; as a result, the first screening was performed with the bacterial viruses P100 and PRD1. The individual liquids consist of a high hydrophobic cation, anion or both. The phase-concentration potential of the liquids was investigated with plaque assay and quantitative real-time polymerase chain reaction (qPCR). Last but not least, the influences of pH, salinity as well as other external inhibitors, such as carbohydrates, proteins and lipids were also determined with the most promising phase-systems.

**Results:** Our results exhibited good separation properties of at least two ILs, whereas most of them also led to the inactivation of the virus particles. The investigation of external factors (like pH, salinity, etc.) exhibited influence on the two-phase-system, which need to be optimized. The results indicated that especially a pH of 7.5 and protein impurities decreased the amount of viral particles in the aqueous phase of both bacterial viruses, P100 and PRD1.

**Conclusions:** All in all, these preliminary results showed promising potential of IL-phase-systems for concentration of viruses.

---

## 234-VPP

### Impacts of prophage encoded genes on host metabolic networks

\*N. Saadat<sup>1</sup>

<sup>1</sup>Heinrich Heine Universität Düsseldorf, Quantitative und Theoretische Biologie, Düsseldorf, Germany

Dynamics of bacterial populations, as well as prokaryotic evolution itself, are strikingly affected by bacteriophages. The integration of the phage genome into the host chromosome as a prophage can be for the host beneficial. Phages arose from a temperate state can pick host genetic material during their replication cycle and introduce it into the new target when getting back into the dormant, prophage state. This mechanism called transduction, enables a putative fitness advantage for the host by acquiring new genes coding for a metabolic function. The impact of these transduced genes to the host fitness is still debated and poor studied systematically. In this study we coupled a bioinformatic approach with a network expansion algorithm and developed a new method that allows to quantify the impact of prophage encoded genes to the bacterial metabolic network. Our results shows that genes with strong impact on the host metabolism are significantly under represented in all prophages along the full bacterial taxonomic range. In some cases we identify reactions encoded in prophage regions having a significantly high impact on the metabolic network. Furthermore, some of these high impact reactions are part of the pentose phosphate pathway or are associated with pentose and nucleotide metabolism. Our method shows that phage domestication towards fitness benefits is reserved just for a minority of bacterial strains.

---

## 235-VPP

UViGs enable the assessment of viral diversity and their importance along the Peruvian coast

\*C. M. Chibani<sup>1</sup>, M. Fischer<sup>1</sup>, A. Kupczok<sup>1</sup>, G. Lavik<sup>2</sup>, R. Schmitz-Streit<sup>1</sup>

<sup>1</sup>Christian-Albrechts-University, Institute of General Microbiology, Kiel, Germany

<sup>2</sup>Max-Planck-Institut für Marine Mikrobiologie, Bremen, Germany

It is widely recognized that viruses play an important role in the biology and evolution of their host and in biogeochemical cycling. Many efforts have been made in the last decade to uncover, classify and expand our knowledge of the earth's virosphere, beyond the limits of viral identification in cell cultures, through culture-independent sequencing. These efforts broadened the number of known viral genera and lead to a substantial increase in the number of uncultivated virus genomes (UViGs).

In this study, we aimed to explore the virosphere of the Oxygen Minimum Zone (OMZ) off the coast of Peru through the *de novo* construction of high-quality reference UViGs. To address this question, 8 different stations were sampled and sequenced, based on various O<sub>2</sub> characteristics, during the cruise of the research vessel Meteor M138 along the Peruvian coast within the upwelling region. The resulting sequences were subjected to pre-processing, filtration, assembly and reference-based and reference-free viral identification pipelines to single out putative marine viral genomes.

Preliminary results indicate the identification of UViGs fitting community-wide adopted standards which we use for accurate taxonomic and functional characterization of viral diversity. Additionally, we expect to find habitat-specific auxiliary metabolic genes within the viral genomes, habitat-type specific viruses as well as ubiquitously found viral groups. Our results emphasize the global viral diversity and provide a detailed insight into viral habitat distribution and host-virus interactions.

---

## 236-VPP

### Isolation and characterization of novel *Streptomyces* phages

\*A. Hardy<sup>1</sup>, L. Kever<sup>1</sup>, V. Sharma<sup>1</sup>, J. Frunzke<sup>1</sup>

<sup>1</sup>Forschungszentrum Jülich, IBG-1: Biotechnology, Jülich, Germany

#### Introduction

Bacteriophages—or phages for short—are viruses which infect bacteria. Phages are the most abundant organisms in the biosphere, with an estimated 10<sup>31</sup> phage particles on our planet. Yet, fewer than 20.000 phage genomes have been described until now—many of which having not been thoroughly characterized. A similar situation applies to *Streptomyces*, a bacterial genus much studied for its complex secondary metabolism. Around 80 phages infecting *Streptomyces* have been described so far; however model phages which have been extensively characterized are lacking.

#### Objectives

We aim at isolating and characterizing novel phages infecting *Streptomyces*.

#### Materials & methods

We isolated phages from soil samples capable of infecting *Streptomyces venezuelae* or *Streptomyces coelicolor*. The DNA of these phages was extracted and subjected to genome sequencing. We assembled the phage genomes

and annotated them to assign putative function to predicted coding sequences.

The novel phages were also imaged by transmission electron microscopy and experimentally characterized in terms of host-range and infection dynamics.

## Results

We isolated from soil samples phages infecting *Streptomyces venezuelae* and *Streptomyces coelicolor*, respectively. Transmission electron microscopy identified all five phages as members of the Siphoviridae family. Their genomes ranged from 39 to 133kb, and their GC content was comprised between 48 and 72%. Lifestyle predictions classified four of them as temperate, one being predicted as lytic. Interestingly, three phages encode homologs of Lsr2 proteins, which are normally used by bacteria to silence foreign DNA. Further genome analyses revealed the pervasiveness of Lsr2-like proteins among phages infecting Actinobacteria, with more than 20% of *Streptomyces* phages encoding such proteins.

## Conclusion

We isolated and characterized five novel *Streptomyces* phages. Three of them feature Lsr2-like xenogeneic silencer proteins, which are the subject of further investigations.

### 237-VPP

#### Phage-Paper-Scissors - A new paper-based sampling method for monitoring of viruses

\*M. Bobal<sup>1</sup>, J. Sommer<sup>1</sup>, P. J. Mester<sup>1</sup>, A. K. Witte<sup>2</sup>, D. Schoder<sup>3</sup>, P. Rossmann<sup>1,3</sup>

<sup>1</sup>Vetmeduni Vienna, CD-MOMIKO, Vienna, Austria

<sup>2</sup>Hygiene Technologie Kompetenzzentrum GmbH, Bamberg, Germany

<sup>3</sup>Vetmeduni Vienna, Unit of Food Microbiology Institute of Food Safety, Food Technology and Veterinary Public Health Department for Farm Animals and Veterinary Public Health, Vienna, Austria

## 1. Introduction

Bacteriophages are increasingly mentioned as a potent measure to overcome hygiene-related issues in food industry as well as antibiotic resistance in medicine. Unfortunately, bacteria also tend to develop resistance against bacteriophages as well and should not be traded as a "miracle cure". Therefore, monitoring of the abundance of bacteriophages, especially of self-introduced ones, in food production areas will be an important indicator to proof the suitability of this new disinfection and therapeutic method.

## 2. Objectives

For surveillance of such bacteriophages, we developed and evaluated a method that is easy to use at low cost to be as sensitive as conventional swabbing systems.

## 3. Materials & methods

(Artificially contaminated) Stickers of plain paper were analyzed using plaque assay and qPCR. As the majority of the bacteriophages used as disinfectant belong to the order of *Caudovirales*, bacteriophage P100 was used as a representative in this first setup.

## 4. Results

After demonstrating the suitability of paper stickers to uptake and release phage P100 over a 14 day time period, additional tests were performed, such as the determination of the recovery rate compared to conventional swabs. Further, we also confirmed the feasibility of pooling, resilience to cleansing and disinfection and experiments under real conditions by sampling of various door handles. Results suggest that the presented sticker system might offer a promising cost effective alternative sampling system with improved handling characteristics.

## 5. Conclusion

As the use of bacteriophages is increasing, especially in food industry, the continuous determination of the bacteriophage load of food surroundings is getting crucial to prevent phage resistance development, phage spread as well as phage persistence. Our investigation for a novel qPCR-based sampling system with highest sensitivity and with long-term monitoring ability, not only offers new possibilities as monitoring measure, but also facilitates the collection of viromics relevant data.

### 238-VPP

#### Expanding viromes associated with *Brevundimonas* and *Serratia*

\*I. Friedrich<sup>1</sup>, B. Bodenberger<sup>1</sup>, A. Klassen<sup>1</sup>, R. Hertel<sup>1</sup>, R. Daniel<sup>1</sup>

<sup>1</sup>Georg-August-Universität Göttingen, Genomic and Applied Microbiology, Göttingen, Germany

It is indicated that viruses of bacteria (phages) use predominantly dsDNA as genomic material and their genomes are highly diverse. To investigate both assumptions new experimental paths need to be taken. We combined isolation-based methods and different general metagenomic approaches. Two new bacterial host systems, *Brevundimonas* and *Serratia* were isolated and characterized. *Brevundimonas*, a k-strategist, with an oligotrophic lifestyle. *Serratia*, is a r-strategist, with a copiotrophic lifestyle. Both strains were used for phage enrichment using water samples of the primary treatment step derived from Göttingen (Germany) sewage plant. In this way, the generated host-based viromes were addressed with classical isolation-based approaches as well as with modern metagenomic approaches targeting putative phages containing dsDNA, ssDNA, dsRNA or ssRNA as genetic material.

### 239-VPP

#### Time series analysis of lysogeny dynamics in different environments

\*E. Oldenburg<sup>1</sup>

<sup>1</sup>Heinrich-Heine Universität Düsseldorf, Institute of quantitative and theoretical biology, Düsseldorf, Germany

It is known that microbial viruses can control host abundance through the density-dependent dynamics of lytic predators and prey. However, not much is known about whether, and if so how, moderate viruses that coexist and multiply with their host affect microbial communities. It has already been shown that virus-like particles are relatively rare at high host densities. This could indicate suppressed lysis. Various established models, for example, have shown that lytic dynamics are preferred. Meta-analyses of published viral and microbial densities showed that such a trend was widespread in different ecosystems, from soil to freshwater to human lungs. To understand in detail how lysogeny dynamics affects the microbial community we investigate

different time series data sets from different environments. In the actual study we combine bioinformatics approaches that characterizes host-associated pro-/phages, with mathematical modeling that allows explaining observed dynamics in the different data sets. Therefore we examined different lysogeny-controlling scenarios, host-triggered (defense mechanism) or phage triggered (arbitrium like mechanisms) or both. Our results shows that a single scenario can't explain observed dynamic in the different datasets and pinpoints to the influence of other factors that are yet not characterized.

## 240-VPP

### Bacteriophages and their secrets: discovering unknown, lytic properties of tail tubular proteins from *Y. enterocolitica* and *K. pneumoniae*-infecting viruses.

\*N. Urbanska<sup>1,2</sup>, E. Brzozowska<sup>1</sup>, A. Pyra<sup>3</sup>, K. Filik<sup>1</sup>

<sup>1</sup>Ludwik Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Department of Immunology of Infectious Diseases, Wrocław, Poland

<sup>2</sup>University of Wrocław, Faculty of Biological Sciences, Institute of Experimental Biology, Wrocław, Poland

<sup>3</sup>University of Wrocław, Faculty of Chemistry, Department of Crystallography, Wrocław, Poland

**Question:** Multi-resistant, biofilm-forming bacteria are prevalent problem in human society. Since the alternatives for antibiotics are running out, researchers' attention has been drawn to bacteriophages. These viruses are a promising alternative for treating bacterial diseases. However, for a large number of phages, it still remains to be discovered which proteins take part in the infection process. Investigating biochemical properties of structural phage proteins is a new and promising approach. The main focus of this research, dual-function tail tubular proteins, are thought to take part not only in stabilizing the bacteriophage structure, but also to display enzymatic activity. The question we addressed was if TTPAgp11 from  $\phi$ YeO3-12 bacteriophage exhibits enzymatic activity towards disaccharide substrates.

**Methods:** Purification was conducted using batch chromatography. Clustal Omega tool was utilized to perform multiple sequence alignments among TTPAs. The substrate binding by TTPAgp11 was verified by thermophoresis, whereas the substrate-dependent stability was analyzed using nanoDSF technology.

**Results:** It was observed that maltose had a weak effect for TTPAgp11 stabilization. The thermophoretic profiles for both TTPAs suggested that TTPA gp11 exhibits the same enzymatic activity as TTPAgp31. Furthermore, it was confirmed that sequence similarity between TTPAs gp31 and gp11 is over 50%.

**Conclusions:** Until recently, tail tubular proteins have been considered as structural elements only. Our data confirms that TTPAs exhibit biochemical activity. Therefore, their ability to hydrolyze disaccharides can be associated with antibacterial properties.

[1]Brzozowska E et al. Hydrolytic activity determination of tail tubular Protein A of *Klebsiella pneumoniae* bacteriophages towards saccharide substrates. J Sci Rep. 2017;7(1):18048.

[2]Pyra A et al. A biochemical features of a novel Tail Tubular Protein A of *Yersinia* phage  $\phi$ YeO3-12 - an antibiofilm agent against antibiotic-resistant pathogenic bacteria. J Sci Rep. (article in submission)

## 241-VPP

### In vitro analysis of O-antigen specific bacteriophage P22 inactivation by *Salmonella* outer membrane vesicles

\*M. S. Stephan<sup>1,2</sup>, N. K. Broeker<sup>1</sup>, A. Saragliadis<sup>3</sup>, D. Linke<sup>3</sup>, S. Barbirz<sup>4</sup>

<sup>1</sup>Universität Potsdam, Physical Biochemistry, Potsdam, Germany

<sup>2</sup>Max-Planck-Institut für Kolloid- und Grenzflächenforschung, Theorie & Bio-Systeme, Potsdam, Germany

<sup>3</sup>Universitetet i Oslo, Institutt for biovitenskap, Blindern, Norway

<sup>4</sup>Universität Potsdam, Physikalische Biochemie, Potsdam, Germany

Bacteriophages use a large number of different bacterial cell envelope structures as receptors for surface attachment. As a consequence, bacterial surfaces represent a major control point for the defense against phage attack. One strategy for phage population control is the production of outer membrane vesicles (OMVs). In Gram-negative host bacteria, O-antigen specific bacteriophages address lipopolysaccharide (LPS) to initiate infection, thus relying on an essential outer membrane glycan building block as receptor that is constantly present also in OMVs. In this work, we have analyzed interactions of *Salmonella* (S.) bacteriophage P22 with different OMV preparations derived from *S. Typhimurium*. OMVs were generated by accumulation of outer membrane after mechanical cell lysis or by isolation of naturally budded OMVs. The different vesicle preparations were characterized according to their composition, morphology and interaction with phage P22. Especially the efficiency of bacteriophage inactivation and DNA ejection in presence of OMVs was studied.

## 242-VPP

### *Pseudomonas aeruginosa* bacteriophages for possible therapeutic applications

\*T. Martens<sup>1</sup>, G. Maschkowitz<sup>1</sup>, S. Schubert<sup>1</sup>, H. Fickenscher<sup>1</sup>

<sup>1</sup>Institute for Infection Medicine, Kiel, Germany

*Pseudomonas* (P.) *aeruginosa* is a highly relevant bacterial pathogen causing, e. g., pneumonia, sepsis, or wound infections. This species is characterized by pronounced and furthermore variable antibiotic resistances. Bacteriophages are a promising additional antimicrobial therapeutic approach.

Using clinical *P. aeruginosa* isolates including multidrug-resistant variants as host organisms, 13 bacteriophages have been isolated from wastewater. After several plaque purifications, the host range was assessed by spot test assays using 130 clinical *P. aeruginosa* isolates, again including multidrug-resistant strains. The results demonstrated that all the tested multidrug-resistant bacteria were susceptible to lytic bacteriophage infection. The isolated phages showed strongly diverse host ranges (23.7 - 62.7 %) including 11 % primarily phage-resistant isolates.

In order to further broaden the host range, single phages were passaged on initially resistant *P. aeruginosa* isolates and new phages were isolated against the primarily phage-resistant strains. A cocktail of five bacteriophages with the broadest host spectrum showed an extensive coverage of the 130 *P. aeruginosa* isolates tested. Thus, a broad-spectrum coverage of diverse *P. aeruginosa* variants for therapeutic purposes seems feasible.

## 243-SCAP

### Studying bacteriophage infections using Raman microspectroscopy

\*I. Monsees<sup>1</sup>, V. Turzynski<sup>1</sup>, A. J. Probst<sup>1</sup>

<sup>1</sup>Institute for environmental microbiology and biotechnology, Chemistry, Essen, Germany

## Introduction

Viral particles were characterized by Raman spectroscopes long before Raman microspectroscopes were commercially available [1]. However, Raman microspectroscopes have only a spatial resolution of approximately 1 mm enabling measurements of single bacterial cells but not of their respective viruses. Since studies have shown that Raman spectra of bacteria allow differentiation of cells of one species between its growth phases [2], we hypothesize that this method can also be used to differentiate between virus-infected cells and uninfected cells.

## Objective

The aim of this work was to find marker Raman shifts to spot viral infections in bacterial pure cultures using multivariate data analysis.

## Material and Methods

*Pseudomonas* sp. DSM 21482 was grown in TSB medium and infected with phage phi 6. Throughout the growth curve, samples for Raman spectroscopy were taken at several time points, fixed, dehydrated and stored at -20 °C until spectral acquisition using a Renishaw inVia™ confocal Raman Microscope. The acquired spectra were analyzed using the R package Micro Raman [3].

## Results

We present the ordination analysis of single microbial Raman spectra for different time points of their growth curve. The hypothesis was tested using a Monte Carlo-based permutation procedure based on the categories of uninfected and infected *Pseudomonas* cells. Additionally, we describe substantial differences between spectra of the two categories.

## Conclusion

Raman spectroscopy is a powerful tool to differentiate uninfected from infected microbial cells and might have high potential for providing key information for detecting viral infections in environmental samples when combined with other microscopy techniques.

[1] Li, T., *et al.* (1993) *Journal of Molecular Biology* **230**(2): 461-472.

[2] Huang, W. E., *et al.* (2004) *Analytical Chemistry* **76**(15): 4452-4458.

[3] García-Timermans, C., *et al.* (2018) *Journal of Microbiological Methods* **151**: 69-75.

## 244-SCAP

### Neutral mechanisms and niche differentiation in steady-state insular microbial communities revealed by single cell analysis

\*Z. Liu<sup>1</sup>, N. Cichocki<sup>1</sup>, T. Hübschmann<sup>1</sup>, C. Süring<sup>1</sup>, I. D. Ofițeru<sup>2</sup>, W. Sloan<sup>3</sup>, V. Grimm<sup>1</sup>, S. Müller<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research GmbH - UFZ, Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Newcastle University, Newcastle upon Tyne, United Kingdom

<sup>3</sup>University of Glasgow, Glasgow, United Kingdom

In completely insular microbial communities, evolution of community structure cannot be shaped by the immigration of new members. Also, when those communities are run in

steady-state, the influence of environmental factors on their assembly is reduced. Therefore, one would expect similar community structures under steady-state conditions. Yet, in parallel setups variability does occur. To reveal ecological mechanisms behind this phenomenon, five parallel reactors were studied at the single-cell level for about 100 generations and community structure variations were quantified by ecological measures. Whether community variability can be controlled was tested by implementing soft temperature stressors as potential synchronizers. The low slope of the log-normal rank-order abundance curves indicated a predominance of neutral mechanisms, i.e., where species identity plays no role. Variations in abundance ranks of subcommunities and increase in inter-community pairwise  $\beta$ -diversity over time support this. Niche differentiation was also observed, as indicated by steeper geometric-like rank-order abundance curves and increased numbers of correlations between abiotic and biotic parameters during initial adaptation and after disturbances. Still, neutral forces dominated community assembly. Our findings suggest that complex microbial communities in insular steady-state environments can be difficult to synchronize and maintained in their original or desired structure as they are non-equilibrium systems.

## 245-SCAP

The author has not agreed to a publication.

## 246-SCAP

Insights into single-cell heterogeneity of prokaryotes by single-cell RNAseq

\*C. Homberger<sup>1</sup>, F. Imdahl<sup>1</sup>, E. Vafadarnejad<sup>1</sup>, A. E. Saliba<sup>1</sup>, J. Vogel<sup>1,2</sup>

<sup>1</sup>Helmholtz-Institute for RNA-based Infection Research (HIRI), RNA Biology of bacterial infections, Wuerzburg, Germany

<sup>2</sup>University of Wuerzburg, Institute for Molecular Infection Biology (IMIB), Wuerzburg, Germany

RNA-sequencing (RNA-seq) technologies have provided much insight into the transcriptional and regulatory networks in many bacterial and host populations. However, these approaches typically report average expression profiles in populations and fail to provide information on the single-cell level. Heterogeneity at the single-cell level has been explored mostly in eukaryotic cells; however, bacterial populations that are genetically identical have also been shown to exhibit dramatic heterogeneity that has been difficult to explore due to the lack of global single-cell approaches. Here, we investigated the heterogeneity of *Salmonella enterica* serovar Typhimurium (*S. enterica*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) under different growth conditions using a poly(A)-independent single-cell RNA-seq (scRNA-seq). We compared three different growth conditions for *S. enterica*: (i) mid-exponential, (ii) late-exponential and (iii) early-stationary phase. *P. aeruginosa* was investigated under anaerobic stress conditions and represents the first data set generated for this species. Our results show a heterogeneous behaviour in cells grown in mid-exponential growth phase compared to the late-exponential as well as early-stationary phase. The results show that our scRNA-seq workflow can be successfully implemented with different bacterial species. One relevant hurdle to take is the inefficient lysis of the different bacterial species. For this purpose, we aim to develop a lysis validation assay in order to apply the scRNA-seq approach to different bacterial species and growth conditions. Having those transcriptomes from single bacterial cells of different species will promise a better understanding of cell-to-cell heterogeneity, persister cell formation and antibiotic resistance mechanisms.

---

## 247-SCAP

### Comparative analyses of plasmodial network growth in three different slime molds (Myxogastria)

\*C. Westendorf<sup>1,2</sup>, C. J. Gruber<sup>2</sup>, K. Schnitzer<sup>2</sup>, S. Kraker<sup>2</sup>, M. Grube<sup>2</sup>

<sup>1</sup>Max Planck Institute for Dynamics and Self-Organization, Goettingen, Germany

<sup>2</sup>University of Graz, Institute of Biology, Graz, Austria

The phaneroplasmodium of Myxogastria is a large single cell and relies on pulsatile contractions to distribute nutrients or signalling molecules across its network. Its most prominent member, *Physarum polycephalum*, has emerged as a model organism to study biological fluid dynamics and foraging behavior. However, Myxogastria comprises of more than 1000 species [1], many of them with an extended phaneroplasmodium during their life cycle. Here we studied the plasmodial growth and network development in two further slime mold species: *Badhamia utricularis* and *Fuligo septica*. After transferring these sampled slime molds to laboratory culture conditions we first recorded their plasmodial expansion and subsequently applied quantitative image analysis to extract the network and oscillatory properties. We discovered significant variations in chemotaxis, velocity, and oscillatory behavior of plasmodia among and within these species. This shows that the phaneroplasmodia of slime molds develop diverse and specifically organized networks [2].

[1] Lado C 2005–2018 An online nomenclatural information system of eumycetozoa (<http://eumycetozoa.com/data/index.php>).

[2] C Westendorf, CJ Gruber, K Schnitzer, S Kraker, M Grube, Journal of Physics D: Applied Physics 51 (34), 344001, 2018.

---

## 248-SCAP

### Noise in virulence gene regulation in *Staphylococcus aureus*

\*S. George<sup>1</sup>, E. Lehmann<sup>1</sup>, A. Duque-Jaramillo<sup>1</sup>, N. Vetter<sup>1</sup>, D. Keinhörster<sup>1</sup>, G. Hermsdorf<sup>2</sup>, E. Schäffer<sup>2</sup>, F. Oesterhelt<sup>1</sup>, C. Wolz<sup>1</sup>

<sup>1</sup>University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany

<sup>2</sup>University of Tübingen, Cellular Nanoscience, Center for Plant Molecular Biology (ZMBP), Tübingen, Germany

Bacteria respond to changing environments by various adaptive strategies. This includes mechanisms leading to phenotypic variability within a genetically homogeneous population. In *Staphylococcus aureus* the synthesis of capsular polysaccharide (CP) was shown to be highly heterogeneous and growth phase dependent, and correlates with expression of the *capA-P* operon coding for biosynthesis genes. The noise in *cap* expression is an example of phenotypic heterogeneity in pathogenic bacteria increasing the adaptive capacity of the population. The temporal nature of *cap* expression also designates it as a stationary phase marker and possibly an indicator of the metabolic status of the cell. The expression of *cap* is regulated at the promoter level by sigma factor B and several transcription factors such as CodY, Rot, Sae. Furthermore, metabolic cues may also impact CP synthesis at the post-transcriptional level. The high degree of noise in CP production may thus be due to noise propagation from the large number of regulators, cell to cell differences in metabolism or the convergence of these determinants.

Here we aim to decipher the primary source of noise. To this end, we established single-cell assays such as

immunofluorescence, promoter-activity microscopy, mRNA fluorescent in-situ hybridisation and live cell imaging within a microfluidic device. We confirmed heterogeneity at the CP synthesis level as well on the transcriptional level in single cells. *Cap* promoter activity occurred only in non-growing bacterial sub-populations and ATP depletion rapidly triggers CP synthesis. Correlation of dual *cap* promoter activities within single cells revealed that noise in *cap* transcription is mainly extrinsic and thus driven by fluctuation in cellular components. We now correlate noise of candidate regulators with that of *cap* gene expression within the same cell under defined environmental stress conditions. This allows us to determine which factor mainly drives heterogeneity of *cap* and potentially other stationary phase genes.

---

## 249-SCAP

### Flow Cytometric Analysis and Quantification of *Clostridioides difficile* and its Spores

\*C. Döring<sup>1</sup>, J. Fischer<sup>1</sup>, H. Bahl<sup>1</sup>

<sup>1</sup>Universität Rostock, Microbiology, Rostock, Germany

The *Clostridioides difficile* infection (CDI) is one of the main causes for nosocomial diarrhoea in the EU, leading to mass outbreaks and high mortality of risk groups. Spores of *C. difficile* are a main factor for infection and recurrence of the disease. Thus, knowledge about spores is essential to develop treatment strategies against CDI. The analysis of spores of different *C. difficile* strains is difficult and time consuming with classical microbiological methods. In contrast, flow cytometry is a method that can analyse a variety of cells within short time and with high precision. Therefore, the objective of this work was to develop a method for the analysis of spores of *C. difficile*. First, spores of strain 630 were purified. Then vegetative cells and purified spore suspensions were stained with a live-dead-staining, containing the fluorescent dyes thiazol orange (TO) and propidium iodide (PI). Samples were then analysed by flow cytometry. These results were compared to spore counts that were obtained by plating or in a counting chamber. Finally, the method was tested by analysing mixed cultures of vegetative cells and spores, that were incubated for up to 96 h. The purification protocol yielded spore suspensions with 97 % purity. Flow cytometric spore quantification of these samples resulted in the same order of magnitude as spore counting. In comparison to vegetative cells, spores showed a difference in their ability to take up fluorescent stains, as vegetative cells displayed TO and PI intensities 10 times higher than spores. In mixed cultures, only spore concentrations higher than  $1 \times 10^6$  spores/ml correlated with spore counting. These data suggest that high counts of cells and cell debris interfere with the spore signal and thus alter the spore count.

We conclude that flow cytometry is suitable to quantify spore concentration in pure suspensions. The analysis of spores in the presence of high cell or cell debris concentrations by flow cytometry needs further optimization.

---

## 250-SCAP

### Quantitative assays for single cell studies of spore germination

\*S. Schwindt<sup>1</sup>, M. Kilian<sup>2,1</sup>, R. Koch<sup>2,1</sup>, B. Steinfeld<sup>2,1</sup>, E.

Kaganovitch<sup>3</sup>, B. Wollenhaupt<sup>3</sup>, D. Kohlheyer<sup>3</sup>, I. Bischofs-Pfeifer<sup>2,1</sup>

<sup>1</sup>MPI for Terrestrial Microbiology, Marburg, Germany

<sup>2</sup>University of Heidelberg, BioQuant, Heidelberg, Germany

<sup>3</sup>Forschungszentrum Jülich, Jülich, Germany

When exposed to nutrient starvation Bacilli forms highly resistant spores which can stay dormant for many years and

revive almost instantly when conditions improve. Spore germination is a fascinating process which plays an important role for the function of many probiotics. Quantitative single cell studies of spore germination are challenging as suitable experimental and bioinformatics analysis tools are lacking. Here we present two experimental assays for a quantitative analysis of germination traits based on time-lapse microscopy by using defined hydrogels and a microfluidic device, respectively. Moreover, an image processing pipeline which segments, tracks and classifies non-germinating and germinating spores was developed. We apply both assays to study the heterogeneous germination dynamics of *B. subtilis* spores in response to L-alanine. Individual spores differ in their propensity for germination in response to specific nutrient germinants and the dynamics of spore germination is also highly variable. The average germination kinetics of spore populations are comparable in both assays. While hydrogel assays have the advantage of generating higher throughput, microfluidic devices provide superior control enabling one to precisely maintain and change environmental conditions. In summary, we developed two complementary set-ups that are a suitable for single cell studies of spore germination.

## 251-MSRP

***Staphylococcus aureus*** responds to allicin by global S-thioallylation - role of the Brx/BSH/YpdA pathway and the disulfide reductase MerA to overcome allicin stress

\*V. L. Vu<sup>1</sup>, T. T. H. Nguyen<sup>1</sup>, T. Busche<sup>1,2</sup>, N. T. Quach<sup>1</sup>, M. C. H. Gruhlke<sup>3</sup>, J. Kalinowski<sup>4</sup>, J. Bernhardt<sup>5</sup>, A. Slusarenko<sup>3</sup>, H. Antelmann<sup>1</sup>

<sup>1</sup>Freie Universität Berlin, Institut für Biologie-Mikrobiologie, Berlin, Germany

<sup>2</sup>Bielefeld University, Center for Biotechnology, Bielefeld, Germany

<sup>3</sup>RWTH Aachen University, Department of Plant Physiology, Aachen, Germany

<sup>4</sup>Bielefeld University, Center for Biotechnology, Bielefeld, Germany

<sup>5</sup>Ernst-Moritz-Arndt-University of Greifswald, Greifswald, Germany

**Introduction:** Allicin (diallyl thiosulfinate) from garlic exhibits broad-spectrum antimicrobial activity against many multidrug resistant bacteria. The thiol-reactive mode of action of allicin involves its S-thioallylations of low molecular weight (LMW) thiols and protein thiols. **Objectives:** We investigated the antimicrobial mode of action and possible detoxification mechanisms of allicin in *S. aureus* USA300. **Materials & Methods:** We used RNA-seq transcriptomics, proteomics, redox biosensor measurements and phenotype analyses to study the stress responses, redox changes and the targets for S-thioallylation by allicin in *S. aureus*. **Results:** Allicin caused a strong thiol-specific oxidative and sulfur stress response and protein damage as revealed by the induction of the PerR, HypR, QsrR, MhqR, CstR, CtsR, HrcA and CymR regulons in the RNA-seq transcriptome. Allicin also interfered with metal and cell wall homeostasis and caused induction of the Zur, CsoR and GraRS regulons. Brx-roGFP2 biosensor measurements revealed a strongly increased EBSH under allicin stress. In the proteome, 57 proteins were identified with S-thioallylations under allicin treatment, including translation factors (EF-Tu, EF-Ts), metabolic and redox enzymes (AldA, GuaB, Tpx, KatA, BrxA, MsrB) as well as redox-sensitive MarR/SarA-family regulators (MgrA, SarA, SarH1, SarS). Phenotype and biochemical analyses revealed that BSH and the HypR-controlled disulfide reductase MerA are involved in allicin detoxification in *S. aureus*. The reversal of protein S-thioallylation was catalyzed by the Brx/BSH/YpdA pathway. Finally, the BSSB reductase YpdA was shown to use S-allylmercaptobacillithiol (BSSA) as substrate to regenerate BSH in *S. aureus*. **Conclusion:** Allicin results in an oxidative shift of EBSH and protein S-thioallylation, which can be reversed by YpdA and the

Brx/BSH/YpdA electron pathways in *S. aureus* to regenerate thiol homeostasis (1).

1. Loi VV et., al. (2019) *Staphylococcus aureus* responds to allicin by global S-thioallylation - Role of the Brx/BSH/YpdA pathway and the disulfide reductase MerA to overcome allicin stress. *Free Radic Biol Med.* 139: 55-69.

## 252-MSRP

**The MhqR regulon confers resistance to quinone-like antimicrobials in *Staphylococcus aureus***

\*V. N. Fritsch<sup>1</sup>, \*S. Wellner<sup>1</sup>, V. V. Loi<sup>1</sup>, T. Busche<sup>1,2</sup>, A. Sommer<sup>1</sup>, K. Tedin<sup>1</sup>, D. Nürnberg<sup>1</sup>, J. Kalinowski<sup>2</sup>, J. Bernhardt<sup>3</sup>, M. Fulde<sup>1</sup>, H. Antelmann<sup>1</sup>

<sup>1</sup>Freie Universität Berlin, Berlin, Germany

<sup>2</sup>Bielefeld University, Center for Biotechnology, Bielefeld, Germany

<sup>3</sup>Universität Greifswald, Department for Microbial Physiology and Molecular Biology, Greifswald, Germany

**Introduction:** *Staphylococcus aureus* is a major human pathogen which can cause life-threatening diseases. During infection, *S. aureus* has to cope with reactive oxygen and reactive electrophilic species (ROS and RES). ROS and RES can cause different post-translational thiol-modifications, such as reversible disulfide formation and irreversible S-alkylations. Quinones have antimicrobial properties and were shown to act as electrophiles and oxidants in bacteria. **Objectives:** Here, we aimed to elucidate the quinone stress response in *S. aureus* [1]. The MhqR regulon was most strongly induced under methylhydroquinone (MHQ) stress in the RNAseq transcriptome. Thus, we were interested in the role of MhqR regulon in the quinone stress response and antimicrobial resistance in *S. aureus*. **Methods:** We used genetic, biochemical and phenotype analyses to characterize the novel quinone-responsive *mhqRED* operon. **Results:** Transcriptional analysis showed that the *mhqRED* operon responds specifically to MHQ and to quinone-like antimicrobials, such as ciprofloxacin. It was further shown, that the non-conserved Cys95 of the MhqR repressor is not required for DNA binding and quinone sensing *in vitro* and *in vivo*. The *mhqR* deletion mutant was resistant to MHQ and other antimicrobials and in long-term infection studies. Interestingly, the *mhqR* mutant showed higher respiratory chain activity and increased ATP levels. Phenotype analysis of the *mhqD* deletion mutant revealed that the carboxylesterase MhqD functions in quinone detoxification. Comparative analysis of another quinone-sensing regulator QsrR showed that the QsrR and MhqR regulons confer independently quinone and antimicrobial resistance in *S. aureus*. **Conclusion:** These studies identified a link between quinone and antimicrobial resistance via the MhqR regulon in *S. aureus*. Our current studies are directed to elucidate the quinone sensing mechanism of MhqR using biochemical and crystal structure methods.

[1] Fritsch et al. (2019): "The MhqR repressor confers resistance to quinone-like antimicrobials in *Staphylococcus aureus*", *Antioxid Redox Signal.* doi: 10.1089/ars.2019.7750.

## 253-MSRP

***Staphylococcus aureus*** uses the bacilliredoxin (BrxAB)/ bacillithiol disulfide reductase (YpdA) redox pathway to defend against oxidative stress under infections

\*N. Linzner<sup>1</sup>, V. V. Loi<sup>1</sup>, V. N. Fritsch<sup>1</sup>, Q. N. Tung<sup>1</sup>, S. Stenzel<sup>1</sup>, M. Wirtz<sup>2</sup>, R. Hell<sup>2</sup>, C. J. Hamilton<sup>3</sup>, K. Tedin<sup>4</sup>, M. Fulde<sup>4</sup>, H. Antelmann<sup>1</sup>

<sup>1</sup>Institute for Biology-Microbiology, Berlin, Germany

<sup>2</sup>Centre for Organismal Studies, Department IV Molecular Biology Plants, Heidelberg, Germany



**Introduction:** *Staphylococcus aureus* has to cope with oxidative stress during infections. The low molecular weight thiol bacillithiol (BSH) is an important ROS defense mechanism of *S. aureus* to maintain the redox homeostasis. Under HOCl stress, BSH forms S-bacillithiolations with proteins, which are reduced by the bacilliredoxins BrxA/B. The NADPH-dependent disulfide reductase YpdA was proposed to function as BSSB reductase [1]. **Objectives:** We studied the role of the bacilliredoxin/BSH/YpdA pathway in *S. aureus* COL under oxidative stress and macrophage infections [2]. **Materials & methods:** We used growth and survival assays, HPLC thiol metabolomics, biosensor measurements, infection assays and biochemical analyses. **Results:** Using HPLC thiol metabolomics, a strongly enhanced BSSB level and a decreased BSH/BSSB ratio were measured in the *S. aureus* ypdA mutant under control and NaOCl stress. Monitoring the BSH redox potential ( $E_{BSH}$ ) with the Brx-roGFP2 biosensor revealed that YpdA is required for regeneration of the reduced  $E_{BSH}$  upon recovery from oxidative stress. Phenotype analyses showed that BrxA and YpdA are required for survival under NaOCl and H<sub>2</sub>O<sub>2</sub> stress *in vitro* and inside murine J-774A.1 macrophages *in vivo*. NADPH-coupled electron transfer assays supported the function of YpdA in BSSB reduction *in vitro*. YpdA acts together with BrxA and BSH in de-bacillithiolation of S-bacillithiolated GapDH. **Conclusion:** The Brx/BSH/YpdA pathway plays a crucial role in BSH redox homeostasis and the oxidative stress defense in *S. aureus*.

## References:

[1] Mikheyeva IV, Thomas JM, Kolar SL, Corvaglia AR, Gaioata N, Leo S, Francois P, Liu GY, Rawat M, and Cheung AL. YpdA, a putative bacillithiol disulfide reductase, contributes to cellular redox homeostasis and virulence in *Staphylococcus aureus*. *Mol Microbiol* 111, 1039-1056. (2019)

[2] Linzner N, Loi VV, Fritsch VN, Tung QN, Stenzel S, Wirtz M, Hell R, Hamilton CJ, Tedin K, Fulde M and Antelmann H. *Staphylococcus aureus* uses the bacilliredoxin (BrxAB)/bacillithiol disulfide reductase (YpdA) redox pathway to defend against oxidative stress under infections. *Front. Microbiol.*, 10, 1355. doi: 10.3389/fmicb.2019.01355 (2019)

## 254-MSRP

The author has not agreed to a publication.

## 255-MSRP

The author has not agreed to a publication.

## 256-MSRP

Comparative genomics and transcriptomics to analyze multicellular development in filamentous fungi

R. Lütkenhaus<sup>1</sup>, S. Traeger<sup>1</sup>, J. Breuer<sup>1</sup>, L. Carreté<sup>2</sup>, A. Kuo<sup>3</sup>, A. Lipzen<sup>3</sup>, J. Pangilinan<sup>3</sup>, D. Dilworth<sup>3</sup>, L. Sandor<sup>3</sup>, S. Pöggeler<sup>4</sup>, T. Gabaldón<sup>2</sup>, K. Barry<sup>3</sup>, I. V. Grigoriev<sup>3</sup>, \*M. Nowrousian<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Department of Molecular and Cellular Botany, Bochum, Germany

<sup>2</sup>Centre for Genomic Regulation, Barcelona, Spain

<sup>3</sup>U.S. Department of Energy Joint Genome Institute, Walnut Creek,

**Questions:** The ability to develop complex multicellular structures evolved several times independently in eukaryotes. Within fungi, complex multicellular structures evolved at least twice and possibly up to eleven times. Fungal multicellular structures are often involved in sexual development, e.g. the fruiting bodies of basidiomycetes and filamentous ascomycetes. Fruiting bodies function in the production and dispersal of sexual spores; however, the molecular mechanisms underlying their formation are insufficiently understood. Previous comparative transcriptomics of fruiting body development in different ascomycetes suggested that there might be a core set of genes that are transcriptionally regulated in a similar manner across species. Conserved patterns of gene expression can be indicative of functional relevance.

**Methods:** In this study, we have sequenced the genome of the Pezizomycete *Ascodesmis nigricans*, and performed comparative transcriptomics of developing fruiting bodies of this fungus, the Pezizomycete *Pyronema confluens*, and the Sordariomycete *Sordaria macospora*.

**Results and Conclusions:** With only 27 Mb, the *A. nigricans* genome is the smallest and most gene-dense Pezizomycete genome sequenced to date. Comparative transcriptomics indicated that gene expression patterns in developing fruiting bodies of the three species are more similar to each other than to non-sexual hyphae of the same species. An analysis of 83 genes upregulated only during fruiting body development in all three species revealed 23 genes with predicted roles in transport processes, and 13 genes with predicted roles in chromatin organization or the regulation of gene expression. Among four candidate genes analyzed by deletion in *S. macospora*, three were shown to be involved in fruiting body formation, including two predicted chromatin modifier genes, confirming that genes with evolutionary conserved expression patterns are promising candidates for functional analysis.

## 257-MSRP

### Proteomic adaptation of *Streptococcus pneumoniae* to the human antimicrobial peptide LL-37

\*P. A. Mücke<sup>1</sup>, S. Maaß<sup>1</sup>, S. Hammerschmidt<sup>2</sup>, D. Becher<sup>1</sup>

<sup>1</sup>University of Greifswald/ Institute of Microbiology, Department of Microbial Proteomics, Greifswald, Germany

<sup>2</sup>University of Greifswald/ Interfaculty Institute for Genetics and Functional Genomics, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

The antimicrobial peptide LL-37 is part of the humane innate immune system and can inhibit bacterial growth by membrane pore formation and the consequent loss of bacterial cell content. In human epithelial layers, like the one of the lung, LL-37 is one of the most prominent antimicrobial peptides (AMPs) and defends the host against aspirated bacteria.

However, several resistance and adaptation strategies are described for bacteria to cationic antimicrobial peptides that could also play a role in the process of pneumococcal infections.

In order to investigate the response of *Streptococcus pneumoniae* to physiological concentrations of LL-37 we stressed pneumococci and analyzed the proteome in comparison to the untreated state via high-resolution mass spectrometry. Indeed, our data indicate that also

pneumococci adapt to LL-37 stress to reduce the destructive impact of the AMP, especially by significant changes in the abundance of cell wall modifying proteins. The inhibition of adaptation mechanisms could potentially sensitize the bacteria to already existing endogenous antibiotics in the human body like LL-37 and thus reduce the high burden of pneumococcal disease including otitis media, sinusitis and pneumonia.

## 258-MSRP

Metabolomic profiling of dormant and reactivating

*Mycobacterium smegmatis* cells

\*V. Nikitushkin<sup>1</sup>, G. Demina<sup>1</sup>, M. Shleeva<sup>1</sup>, A. Kaprelyants<sup>1</sup>

<sup>1</sup>Russische Akademie der Wissenschaften, Föderales Forschungszentrum "Biotechnologie"; A.N. Bach Institut für Biochemie, Moskau, Russian Federation

**Introduction.** Under stress conditions (acidification of the culture medium) mycobacterial cells transit into a specific state characterised by low level of metabolic activity-*dormancy*. This state is directly linked to the physiological drug resistance, complicating the control over latent TB. In order to find new anti-LTB compounds the metabolic processes which may occur in dormancy as well as in reactivation, should be characterised. **Methods.** In the current study we analysed the untargeted metabolomic profiles of dormant and reactivating *Mycobacterium smegmatis* cells (a model microorganism mimicking MTB), on the global scale level, since such analysis of metabolites' dynamics could provide an overview on biochemical perturbations as a response to stress conditions. The reactivation process was tracked by measuring the value of transmembrane potential (ratiometric approach based on flow-cytometry). The crucial time-points: 0 hours (dormant cells), 10 hours (reactivating cells) and 26 hours (actively proliferating cells) were selected and sampled to LC-MS profiling. The metabolomic data were statistically evaluated (PCA, hierarchical clustering, differential analysis) and the succession of the metabolic pathways activation was analysed. **Results.** In total, 707 compounds were discovered and analysed. The most significant changes occurred between the timepoints 10 and 26 hours. 77 Metabolites were significantly changed with a global adjusted *p*-value <0.05. Many lipids show a constant increase over all timepoints, e. g. PI(36:1) and PI(P-16:0/15:1(9Z)). However most of the significantly changed metabolites decrease over the analysed period, e. g. isopentenyl pyrophosphate or palmitate. A few metabolites are interestingly increased at 10 hours and decrease again to 26 hours (phosphatidylglycerol, ganoderic acid and some unknown compounds). **Conclusion.** Despite the long storage of dormant cells in the conditions of minimum metabolic activity, the cells still retained a large number of metabolites, demonstrating interesting biochemical patterns of changes in reactivation. In this process a gradual, incremental assembly of complete biochemical pathways could be observed.

## 259-MSRP

A novel approach towards developing an efficient and self-renewing filter system

\*A. Vaishampayan<sup>1</sup>, R. Ahmed<sup>2</sup>, O. Wagner<sup>2</sup>, A. de Jong<sup>3</sup>, C. Meyer<sup>4</sup>, D. Wight<sup>2</sup>, R. Haag<sup>2</sup>, U. Landau<sup>4</sup>, J. Kok<sup>3</sup>, E. Grohmann<sup>1</sup>

<sup>1</sup>Beuth Hochschule fuer Technik Berlin, Berlin, Germany

<sup>2</sup>Free University of Berlin, Berlin, Germany

<sup>3</sup>University of Groningen, Groningen, Germany

<sup>4</sup>Largentec GmbH, Berlin, Germany

Multidrug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) are known to cause severe

nosocomial infections. Around 50,000 people in Europe, die due to nosocomial infections every year. Pathogens like *Legionella* contaminate water, for instance in the sanitary areas. More than 700 million people do not have access to clean drinking water and a total of 1.8 billion people get their drinking water from sources that are contaminated at least temporarily with fecal matter (1). Thus, there is a need to develop an effective and self-renewing filter system for long term use to provide potable water. We aim to develop a universal filter system to diminish the microbial load in water. It will be a depth filter consisting of two components, component 1, which is functionalized graphene oxide (GOX) that binds free microorganisms. Component 2 consists of a carrier material, which is coated with the antimicrobial surface AGXX. Reactive oxygen species are formed on component 2 which kill the microorganisms present in the system. We developed and tested several filter materials consisting of GOX and AGXX using confocal microscopy, growth inhibition, and filtration assays to assess their efficiency. To understand the mechanism of GOX and AGXX, and to determine the effect of these materials on MRSA, we exposed MRSA to GOX, AGXX, or the combination of both and investigated its response via total RNA sequencing. Our microbiological and transcriptomic data indicate that the combination of GOX and AGXX is more efficient than the individual components. GOX-AGXX is a potential antimicrobial and may also inhibit biofilm formation in MRSA by repressing genes related to biofilm formation and virulence, antibiotic resistance, and metabolic genes crucial for biofilm survival.

## References

1. WHO report "Progress on Sanitation and Drinking Water" - 2015 update and MDG assessment (ISBN: 9 789241 509145)

## 260-MSRP

Adjusting ectoine biosynthesis: Crystallographic and functional analysis of the EctR regulator

\*L. Czech<sup>1,2</sup>, B. Frieg<sup>3,4,5</sup>, S. Kobus<sup>6,7</sup>, A. Höppner<sup>6,7</sup>, J. Reiners<sup>6,7</sup>, H. Gohlke<sup>3,4,5</sup>, S. Smits<sup>6,7</sup>, E. Bremer<sup>1,2</sup>

<sup>1</sup>Philipps-University Marburg, Laboratory for Microbiology, Marburg, Germany

<sup>2</sup>Philipps-University Marburg, SYNMIKRO - Center for Synthetic Microbiology, Marburg, Germany

<sup>3</sup>Heinrich-Heine University, Institut für Pharmazeutische und Medizinische Chemie, Düsseldorf, Germany

<sup>4</sup>Forschungszentrum Jülich GmbH, Institute for Complex Systems - Structural Biochemistry (ICS 6), Jülich, Germany

<sup>5</sup>Forschungszentrum Jülich GmbH, John von Neumann Institute for Computing (NIC) & Jülich Supercomputing Centre (JSC), Jülich, Germany

<sup>6</sup>Heinrich-Heine University Düsseldorf, Center for Structural Studies, Düsseldorf, Germany

<sup>7</sup>Heinrich-Heine University Düsseldorf, Institute of Biochemistry, Düsseldorf, Germany

Upon osmotic stress, members of all three domains of life were found to produce the cytoprotectants and chemical chaperones, ectoine and 5-hydroxyectoine, to counteract the osmotically instigated outflow of water. The expression level of the underlying biosynthetic genes [*ectABC(D)*] increases in response to increases in the external salinity and must be tightly controlled to avoid wasteful production of ectoines. Here, we focus on the MarR-type regulator EctR<sup>1</sup>, found in 22% of putative ectoine producing bacteria<sup>2</sup>. Phylogenetic analysis revealed a rather restricted occurrence of *ectR* in the vicinity of ectoine gene clusters of Proteobacteria. Using EctR from the Alphaproteobacterium *Novosphingobium* sp. LH128, we obtained a crystal structure at 2.2 Å resolution. Structural and biochemical analysis revealed an unexpected

tetrameric assembly of two head-to-head homodimers positioning the winged-helix-turn-helix DNA-binding domains at the outside of the protein complex. The *ectR* gene of *Novosphingobium* sp. LH128 is located upstream and divergent of the *ect* gene cluster. *In vitro* binding analysis revealed two individual binding regions of EctR upstream of the hypothetical sigma-70-type promoter of *ectR* and downstream of the potential sigma-70-type promoter in front of *ectA*. Inspection of these regions highlight two pseudopalindromic sequences as potential binding sites for the EctR regulator. Molecular dynamics simulations revealed that the DNA-binding domains of EctR are highly flexible and structural changes within the regulatory protein might be triggered by fluctuations in the ionic potential/strength.

1. Mustakhimov, I. I. *et al.* Identification and characterization of EctR1, a new transcriptional regulator of the ectoine biosynthesis genes in the halotolerant methanotroph *Methylobaculum alcaliphilum* 20Z. *J. Bacteriol.* **192**, 410–417 (2010).
2. Czech, L. *et al.* Role of the extremolytes ectoine and hydroxyectoine as stress protectants and nutrients: genetics, phylogenomics, biochemistry, and structural analysis. *Genes (Basel)* **9**, 177. doi: 10.3390/genes9040177 (2018).

## 261-MSRP

### Cellular response to osmotic challenges of the potential industrial workhorse *Bacillus methanolicus*

\*C. Frank<sup>1</sup>, T. Hoffmann<sup>1</sup>, M. F. Felle<sup>2</sup>, E. Bremer<sup>1</sup>

<sup>1</sup>Philipps-University Marburg, Microbiology, Marburg, Germany

<sup>2</sup>BASF, White Biotechnology RBW, Ludwigshafen, Germany

The Gram-positive thermophilic methylotrophic bacterium *Bacillus methanolicus* was originally isolated from freshwater marsh soil. Due to its ability to utilize methanol as sole carbon- and energy source, *B. methanolicus* is currently explored as a cell factory for the low- cost production of fine chemicals. Its ability to grow at high temperature (50°C) provides benefits to both low contamination risks during fermentation and reactor cooling demands. During high cell density fermentation processes, the excretion of metabolites will increase the osmolarity of the growth medium, which in turn could impair cell growth and negatively affect the productivity of biotechnological processes.

With this in mind, we have analyzed the core of the osmstress response of *B. methanolicus* through physiological approaches. By using natural- abundance <sup>13</sup>C-nuclear magnetic resonance spectroscopy and high-performance liquid chromatography, we discovered that glutamate is synthesized as the sole osmstress protectant by *B. methanolicus*. The glutamate levels increased concomitant with increases in the salinity of the growth medium. Glutamate provided only a moderate level of osmstress protection to *B. methanolicus* when compared to *B. subtilis*, which synthesizes proline as its main osmstress protectant. Strikingly, most of the newly synthesized glutamate by *B. methanolicus* was excreted. Curiously, none of the compatible solutes that can be imported and used effectively by *B. subtilis* as osmstress protectants was able to provide osmstress protection to *B. methanolicus*. Overall, our data indicate that *B. methanolicus* is a rather osmotically sensitive member of the *Bacilli* and possesses only a restricted ability to cope with osmotic stress.

## 262-MSRP

### Beyond damage repair: Cell envelope stress resistance in *Haemophilus influenzae* as a determinant of successful host interactions

M. Nasreen<sup>1</sup>, J. Hosmer<sup>1</sup>, R. Dhoubi<sup>1</sup>, A. T. Essilfie<sup>1</sup>, A. G. McEwan<sup>1</sup>, \*U. Kappler<sup>1</sup>

<sup>1</sup>The University of Queensland, School of Chemistry and Molecular Biosciences, St Lucia, Australia

Many chronic and acute diseases of the respiratory tract are either caused or exacerbated by infections with non-typeable *Haemophilus influenzae* (NTHi), a WHO priority pathogen requiring immediate further action due to increasing disease severity and antibiotic resistance. NTHi thrives at sites of infection, and here we have investigated the molecular mechanisms underlying the remarkable resistance of *H. influenzae* to host-produced bactericidal molecules such as hypochlorite.

To overcome cell envelope stress caused by hypochlorite, all *H. influenzae* strains contain 2 to 3 highly conserved, periplasmic S-oxide reductases that are expressed following hypochlorite exposure and form a first line of defense against cellular damage. The loss of any one of these sulfoxide reductases reduced *H. influenzae* fitness in *in vivo* and also in TC culture infection models despite very different roles in protection from S-oxide stress.

The non-traditional, Mo -containing S-oxide reductase MtsZ reduced free methionine-sulfoxide (MetSO) to Met, thus preventing uptake of MetSO and its incorporation into newly synthesized proteins. The reaction of MtsZ is linked to the Hi respiratory chain, allowing it to be used for energy generation and redox balancing.

In contrast, the MsrAB S-oxide reductase is required for Hi hypochlorite resistance, and repairs MetSO residues in HOCl-damaged proteins which includes key *H. influenzae* OMPs that are highly susceptible to HOCl induced damage.

Unexpectedly, a loss of the MsrAB protein led to specific changes in the host response to infection with NTHi. Increased production of AMPs such as cathelicidin (9x) and BPI (4x) was observed, while expression of two key antiapoptotic proteins, XIAP and BIRC3 was reduced. Thus the conserved extracellular NTHi S-oxide reductases not only prevent and repair HOCl induced damage, but also have previously undocumented links to key virulence relevant processes such as manipulating host responses and successful energy generation.

## 263-MSRP

### Insights into the control of *nlpC*-operon expression in *C. glutamicum* by the two-component system MtrAB and a c-di-AMP responsive riboswitch

\*T. Lkhaasuren<sup>1</sup>, S. Reich<sup>1</sup>, T. Weiss<sup>2</sup>, G. M. Seibold<sup>2</sup>

<sup>1</sup>Ulm University, Institute of Microbiology and Biotechnology, Ulm, Germany

<sup>2</sup>DTU Bioengineering, Department of Biotechnology and Biomedicine, Kgs. Lyngby, Denmark

Cell wall hydrolases of the NlpC/P60 superfamily are required for the reorganization of the bacterial cell wall during vegetative growth, development, and cell division. In Gram-positive model organism *Corynebacterium glutamicum* the genes for two NlpC/P60 endopeptidases involved in cell growth and cell separation are part of an operon (*nlpC*-operon) with further genes for cell wall and sugar metabolism. Upstream of the *nlpC*-operon a cyclic-di-AMP riboswitch and two binding sites for the stress-related

response regulator MtrA are located, suggesting a tight control of this operon. However, physiological studies on the control of *nlpC* by the two-component system (TCS) MtrAB in *C. glutamicum*  $\Delta$ mtrAB were strongly biased, as this deletion mutant has a severe growth defect coming along with an acidification of the medium, which could not be restored by reintroduction of *mtrAB*.

We constructed and then characterized the new *mtrAB*-deficient strain *C. glutamicum*  $\Delta$ mAB, which shows an altered cell shape indicating a change in cell separation. The altered cell separation of  $\Delta$ mAB, which came along with increased transcript levels of the MtrA-controlled *mepA* (encodes a peptidase), was restored upon plasmid-encoded expression of *mtrAB*. Growth of *C. glutamicum*  $\Delta$ mAB in different media proceeded similar to WT. In difference to *C. glutamicum*  $\Delta$ mtrAB, no acidification of the culture medium in the course of cultivation of  $\Delta$ mAB was detected. As expected, no increased transcription of *proP* (encodes the transporter for uptake of the compatible solutes proline and ectoine) was detected in the course of osmotic upshift experiments for *C. glutamicum*  $\Delta$ mAB; induction of *proP* transcription upon osmotic upshift was restored by plasmid-encoded expression of *mtrAB*. For *nlpC* we observed high transcript amounts and promoter activities for WT and increased transcript amounts and promoter activities for  $\Delta$ mAB. These results confirm that MtrA acts as a repressor for transcription of the *nlpC*-operon. The new *mtrAB*-deficient strain *C. glutamicum*  $\Delta$ mAB allows now a detailed analysis of the stimuli underlying the interplay of the TCS MtrAB and the c-di-AMP dependent riboswitch for the control of *nlpC*.

#### 264-MSRP

##### **A Respiratory Supercomplex Links Oxygen and Nitrate Reduction in the Obligate Aerobic Actinobacterium *Streptomyces coelicolor* A3(2)**

\*M. Fischer<sup>1</sup>, \*D. Falke<sup>1</sup>, A. Haase<sup>1</sup>, A. Kieler<sup>1</sup>, C. Ihling<sup>2</sup>, A. Sinz<sup>2</sup>, G. Sawers<sup>1</sup>

<sup>1</sup>Martin-Luther-University, Institute of Biology/Microbiology, Halle, Germany

<sup>2</sup>Martin-Luther-University, Institute of Pharmacy, Halle, Germany

Respiratory supercomplexes are common in aerobic and facultative anaerobic microorganisms and their formation is thought to facilitate efficient coupling of electron flow with proton-translocation by redirecting electrons to different terminal electron-accepting complexes. As an obligate aerobic, filamentous soil-dwelling and spore-forming actinobacterium *Streptomyces coelicolor* A3(2) synthesizes a menaquinol:cytochrome *bcc* oxidoreductase (*bcc* complex)-copper-aa<sub>3</sub>-type cytochrome *c* oxidase supercomplex because, like other actinobacteria, *S. coelicolor* lacks a soluble *c*-type cytochrome. Despite requiring oxygen for growth, *S. coelicolor* can also use nitrate as electron acceptor. The three respiratory nitrate reductases (Nars) required to reduce nitrate are synthesized and active in different stages of the life-cycle. Surprisingly, our data revealed that spore-specific Nar1 requires the *bcc*-aa<sub>3</sub> supercomplex for activity and that mycelium-specific Nar2 activity is also partially dependent on the supercomplex. Using a combination of genetics, protein purification, enzyme activity determination and mass spectrometry we could show that the composition of the *bcc*-aa<sub>3</sub>-supercomplex differs in spores and mycelium. We could also demonstrate that Nar2 interacts directly with components of the *bcc*-aa<sub>3</sub>-supercomplex in mycelium. Together, our findings suggest that, when oxygen becomes limiting, the bacterium can switch immediately to nitrate respiration by redirecting the electrons from the supercomplex to Nar, thus maintaining

efficient proton translocation under oxygen-limiting conditions.

#### 265-MSRP

##### **Global Analysis of Changing Transcript Stability in the Acclimation to Low Iron in a Photosynthetic Cyanobacterium**

\*L. Youssar<sup>1</sup>, G. Kostova<sup>2</sup>, W. R. Hess<sup>1</sup>, J. Georg<sup>1</sup>

<sup>1</sup>Uni-Freiburg, Freiburg im Breisgau, Germany

<sup>2</sup>CNRS, Paris, France

The regulation of RNA stability is crucial in the post-transcriptional regulation of gene expression in bacteria. Here, we determined RNA half-lives in the model cyanobacterium *Synechocystis* sp. PCC 6803 during standard conditions and iron-deplete growth, a condition that poses substantial physiological challenges to a photosynthetic organism and involves well-studied transcriptional and post-transcriptional regulatory mechanisms. We found 3200 transcripts with half-life detected in iron stress conditions while 2567 were in the standard conditions. In total, 2315 different transcripts were detectable under both conditions and subjected to comparative analysis. The median half life was around 2 minutes in both conditions. The results allow for the first time to distinguish between the effects of transcriptional and post-transcriptional regulation in the iron starvation response. Statistical and clustering analyses will aid our understanding of mRNA decay in iron stress condition and further illuminate more about the complexity in the iron regulatory network of cyanobacteria.

#### 266-MSRP

##### **Investigation of the plasma-induced activation of *Escherichia coli* Hsp33**

\*T. Dirks<sup>1</sup>, M. Krewing<sup>1</sup>, J. Bandow<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Applied Microbiology, Bochum, Germany

Cold atmospheric pressure plasmas have a variety of different applications. They are used e.g. in clinical settings for surface decontamination or disinfection [1]. Among the mechanisms underlying bacterial inactivation by plasma, protein aggregation has been shown to be an important one [2,3].

To investigate a potential role of aggregation-preventing proteins in mediating plasma resistance, a quantitative CFU-based assay was used to compare survival of plasma treatment of wild-type *E. coli* to that of deletion mutants and over-production strains. All of the five different aggregation-preventing proteins investigated (Hsp33, DegP, CnoX, RidA, and ClpB) contribute to plasma resistance.

The redox-regulated molecular chaperone Hsp33 has previously been shown to positively impact on plasma survival and to be activated by plasma *in vitro* [3]. Here, using a clinically relevant plasma source (dielectric barrier discharge of Cinogy), we investigated the plasma-induced Hsp33 activation. To this end, Hsp33 activity was determined using a citrate synthase assay, in which active Hsp33 prevents the aggregation of citrate synthase. Oxidation of cysteines and unfolding of the protein, both thought to be crucial for Hsp33 activation, were confirmed using a DTNB assay and CD spectroscopy, respectively. The magnitude of Hsp33 activation by plasma was comparable to chemical activation with HOCl. To gain insights into chemical species relevant for plasma-induced Hsp33 activation, different reactive oxygen species were tested for their ability to

activate Hsp33 *in vitro*, revealing that superoxide, singlet oxygen, and atomic oxygen are potential activators.

Overall, the importance of protein aggregation for plasma-based bacterial inactivation was further corroborated, since we found that aggregation-preventing proteins like chaperones or proteases play a key role in enduring plasma stress.

[1] Ehlbeck *et al.* (2010) *J. Phys. D: Appl. Phys.* **44**, 013002.

[2] Itook *et al.* (2018) *Appl. Microbiol. Biotechnol.* **102**(5), 2279-2288

[3] Krewing *et al.* (2019) *J. R. Soc. Interface* **16**, 20180966.

---

## 267-MSRP

The Cup determinant is crucial for the resistance to copper in *Cupriavidus metallidurans*

\*N. Hirth<sup>1</sup>, M. S. Gerlach<sup>1</sup>, C. Große<sup>1</sup>, D. H. Nies<sup>1</sup>

<sup>1</sup>Martin-Luther-University Halle, Halle (Saale), Germany

In its natural habitat *C. metallidurans* is often exposed to high concentrations of different heavy metals such as copper, gold or mercury. To survive in this toxic environment it has developed tools for detoxification. Here, mutants carrying multiple deletions in copper resistance genes were constructed and used to study the individual contribution of each system to copper and gold resistance. Deleted were operons encoding for the periplasmic copper oxidase *copABCD*, the P-Type-ATPase *cupCAR*, which is located in the inner membrane, the periplasmic spanning efflux pump *cusABC* as well as the *gig*-cluster whose function is yet unclear. A total of 14 multiple mutants was created in the AE104 wildtype background and their respective survival under copper and gold stress was tested. Preincubation with gold or copper led to lower gold resistance in all strains. When exposed to copper the Cup determinant was crucial for survival. So far CupA is the only known protein to detoxify the cytoplasm from copper. A triple mutant of the Cop, Cup and Cus clusters only containing the cup determinant was more resistant to copper than the other triple mutants. Copper or gold preincubation led to higher or lower resistance to copper respectively. The Cus system was not able to compensate for the loss of *cup*. The periplasmic copper oxidase CopA seemed to be more important for the detoxification of the periplasm than the efflux system Cus in a single mutant background. Additional disruption of the gene *gshA* ( $\gamma$ -glutamylcysteine synthetase) led to lower copper resistance which underlines the importance of glutathione in the detoxification process of copper.

1Wiesemann N, Bütof L, Herzberg M, Hause G, Berthold L, Etschmann B, Brugger J, Martinez-Criado G, Dobritsch D, Baginsky S, Reith, F, Nies DH. 2017. Synergistic toxicity of copper and gold compounds in *Cupriavidus metallidurans*. *Appl Environ Microbiol* 83:e01679-17.

---

## 268-MSRP

Work horse strain *Clostridioides difficile* 630 $\Delta$ erm is oblivious to its anaerobic lifestyle

\*D. Troitzsch<sup>1</sup>, H. Zhang<sup>2</sup>, S. Dittmann<sup>1</sup>, D. Dusterhöft<sup>3</sup>, J. M. Borrero de Acuna<sup>2</sup>, S. Sievers<sup>1</sup>

<sup>1</sup>University of Greifswald, Institute of Microbiology, Greifswald, Germany

<sup>2</sup>Technical University of Braunschweig, Institute of Microbiology, Braunschweig, Germany

<sup>3</sup>University of Leipzig, Institute of Biochemistry, Leipzig, Germany

## Introduction

*C. difficile* is a vast problem in human health care, as it causes serious and recurrent inflammation of the intestinal epithelium often with a lethal outcome. It is considered to be an anaerobic bacterium, therefore the presence of oxygen and reactive oxygen species should hamper its growth. However, previous studies have shown a high, strain-dependent oxygen tolerance of the pathogen [1].

## Objectives

To understand the high tolerance of *C. difficile* 630 $\Delta$ erm to oxidative conditions we investigated the structure and function of transcriptional repressor PerR, which is related to the oxidative stress response.

## Materials & Methods

We used different bioinformatic tools to analyze the sequence and structure of PerR in various species and in *C. difficile* 630 $\Delta$ erm specifically. Transcriptional expression of *perR* and growth in the presence of oxygen was tested in both *C. difficile* strains 630 and 630 $\Delta$ erm. Furthermore, DNA-PerR-interaction was examined in electrophoretic mobility shift assays (EMSA).

## Results

Bioinformatic analyses revealed a SNP in the *perR* gene of *C. difficile* 630 $\Delta$ erm, leading to an amino acid exchange of a highly conserved threonine to alanine at position 41 in the protein [2]. Growth experiments showed a significant higher susceptibility of strain 630 to O<sub>2</sub> compared to its derivative 630 $\Delta$ erm. Transcription of the PerR-controlled *rbr1* operon was inducible by H<sub>2</sub>O<sub>2</sub> in strain 630 by a factor of 9, whereas transcript levels in 630 $\Delta$ erm were permanently very high and almost not inducible by H<sub>2</sub>O<sub>2</sub>. EMSA demonstrated a clear DNA-PerR-interaction for *C. difficile* 630, whereas PerR of strain 630 $\Delta$ erm was not capable of binding the DNA fragment of the *rbr1* promoter.

## Conclusion

The laboratory reference strain 630 $\Delta$ erm of the anaerobic human pathogen *C. difficile* is characterized by a remarkable high oxygen tolerance. We showed that an amino acid exchange in the DNA binding domain of the hydrogen peroxide sensor PerR results in a constitutive derepression of PerR-controlled genes. Researchers working with *C. difficile* 630 $\Delta$ erm should be aware of the permanent oxidative stress response even under anaerobic conditions in this reference strain.

[1] Edwards, A. N. et al.; *Front. Microbiol.*, 2016

[2] van Eijk, E. et al.; *BMC genomics* 16, 31 (2015)

---

## 269-MSRP

Spatiotemporal dynamics of the one-component signaling system CadC

\*J. Schwarz<sup>1</sup>, S. Brameyer<sup>1</sup>, E. Hoyer<sup>1</sup>, K. Jung<sup>1</sup>

<sup>1</sup>Ludwig-Maximilians-Universität München, Department of Biology I, Microbiology, Martinsried, Germany

Prokaryotes developed various sensing and signaling mechanisms to withstand environmental stress. The ToxR-

receptor family represents an important member of the one-component signaling systems and is involved in virulence and acid stress response. ToxR-receptors belong to the large group of proteins found in all domains of life that are anchored in the cytoplasmic membrane by one transmembrane helix. Almost nothing is known about their spatiotemporal dynamics in whole cells. One member of this family is CadC, a pH-responsive receptor present in various genera. It has a conserved modular structure with a C-terminal periplasmic sensor domain, a single transmembrane helix and an N-terminal cytoplasmic winged helix–turn–helix DNA-binding domain. Under acid stress, CadC binds to the DNA and induces expression of *cadA* (lysine decarboxylase) and *cadB* (lysine/cadaverine antiporter). It has been shown that fluorophore-tagged CadC is randomly distributed in the membrane of an *Escherichia coli* cell at physiological pH. When the receptor is activated by acid stress, CadC is detectable as a distinct cluster. Thereby the localization of this low copy number membrane-anchored transcription factor is directed by binding to the DNA in a diffusion-and-capture mechanism (1).

To further investigate the dynamics of CadC, the DNA-binding site was relocated in the chromosome of *E. coli*, and the expression pattern as well as the localization of CadC and DNA-locus were studied. In addition, the studies were expanded for CadC in *Vibrio campbellii*, and a ChIP-Seq analysis revealed potential new CadC-binding sites highlighting the common and different aspects in the pH-stress response of various organisms.

(1) Brameyer, S., Rösch, T.C., Andarib, J.A., Hoyer, E., Schwarz, J., Graumann, P.L., Jung, K. (2019) DNA-binding directs the localization of a membrane-integrated receptor of the ToxR family. *Commun. Biol.*, 2:4.

## 270-MSRP

### Inhibition of ribosome rescue mechanisms leads to changes in metal homeostasis in bacteria

\*M. Vázquez-Hernández<sup>1</sup>, J. Bindow<sup>1</sup>, J. Alumas<sup>2</sup>, K. Keiler<sup>2</sup>

<sup>1</sup>Ruhr-Universität Bochum, Applied Microbiology, Faculty of Biology and Biotechnology, Bochum, Germany

<sup>2</sup>Pennsylvania State University, Philadelphia, PA, United States

Protein biosynthesis is the most energy consuming process in bacteria, meaning that at least 50% of the energy consumption of growing bacteria is allocated to translation. It is estimated that per cell cycle 5% of all ribosomes get stalled during translation. Mechanisms to release stalled ribosomes are essential for growth and survival. To date, five ribosome rescue mechanisms are known: *trans*-translation, ArfA and ArfB, ArfT and ResQ. *Trans*-translation and, more generally, ribosome rescue have been proposed as a new antibiotic target. The impact of ribosome rescue inhibition on bacterial physiology is poorly understood. Specific inhibitors of *trans*-translation belonging to different chemical classes have been identified. Six of these compounds were used to study the proteomic response to *trans*-translation inhibition in *E. coli*  $\Delta$ tolC and *B. subtilis* 168. 2D-gel based proteomics was used in conjunction with pulse-labelling to identify differentially regulated proteins. This study was complemented with a gel-free, label-free quantitative LC-MS/MS analysis. Surprisingly, we observed an upregulation of proteins related to copper homeostasis and protection of iron-sulfur clusters. We also found that in *E. coli*, copper supplementation protects bacteria most of the inhibitors, while in *B. Subtilis* copper and inhibitors act synergistically. Pulse-labelling followed by 2D-gel based proteomics performed after exposing the strains simultaneously to copper and inhibitors. In *E. coli*, the simultaneous exposure resulted in the differential regulation of a number of

additional proteins, *B. subtilis*, however, translation was completely inhibited at inhibitor concentrations that by themselves had only minor impact on translation rates. Further experiments will be directed at elucidating the connection between *trans*-Translation and copper homeostasis which could aid in the development of *trans*-translation inhibitor-based therapies and the understanding of antimicrobial resistance.

## 271-MSRP

### Reconstitution of the functional Clp protease complex of streptomycetes *in vitro*

\*L. Reinhardt<sup>1</sup>, D. Thomy<sup>1</sup>, J. Hauser<sup>2</sup>, J. Ortega<sup>3</sup>, P. Sass<sup>1</sup>, H. Brötz-Oesterhelt<sup>1</sup>

<sup>1</sup>University of Tübingen, Department of Microbial Bioactive Compounds, Tübingen, Germany

<sup>2</sup>University of Tübingen, Department of Infection Biology, Tübingen, Germany

<sup>3</sup>McGill University, Department of Anatomy and Cell Biology, Montreal, Canada

Bacterial Clp proteases are multimeric degradation machines that play a major role in the bacterial protein stress response. In principle, a proteolytic core composed of 14 ClpP monomers associates with hexameric Clp-ATPases to degrade misfolded and specific regulatory proteins [1, 2]. Compared to most eubacteria comprising one or two ClpP homologs, streptomycetes rely on a more complex and essential Clp system, involving up to five distinct ClpP homologs organized in two bicistronic and one monocistronic operon [3]. So far, knowledge on the molecular composition, interaction and operation mode of the Clp system in streptomycetes is rare, and a functional Clp system has not been reconstituted *in vitro*.

In this study, we functionally reconstituted the Clp protease complex of *Streptomyces hawaiiensis* NRRL 15010, the producer of the antibacterial complex A54556 [4]. The *clpP* genes of *S. hawaiiensis* show high sequence similarity to other streptomycetes [4]. To shed light on the interaction and functional cross-talk between the ClpP proteins in *Streptomyces*, we expressed and purified the Clp proteins of *S. hawaiiensis* for functional studies using different Clp-ATPases as mediators for substrate degradation by different ClpP isoforms. In addition, ADEP1, the main component of complex A54556 that is known to deregulate ClpP in other bacteria [1, 4], was used to investigate its impact on *Streptomyces* ClpP activity in the natural context. Our data reveals the operation mode of the Clp protease in streptomycetes, bacteria with highly complex physiology that is illustrated by the large number of secondary metabolites, which is an important step to better understand protein homeostasis and protein stress responses guided by the Clp protease in this genus.

1. Brötz-Oesterhelt *et al.* (2005) *Nat Medicine*, 11 (10): 1082-1087
2. Gersch *et al.* (2015) *Nat Commun*, 6: 6320
3. Gominet *et al.* (2011) *Microbiology*, 157: 2226-2234
4. Thomy *et al.* (2019) *Appl Environ Microbiol*, 85 (20): e01292-19

## 272-MSRP

### N-chloramines: reduction mechanism and labeling technique

\*M. Varatnitskaya<sup>1</sup>, K. Xie<sup>1</sup>, K. Rosowski<sup>2</sup>, N. Lupilov<sup>1</sup>, B. Sitek<sup>2</sup>, L. I. Leichert<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Institute of Biochemistry and Pathobiochemistry, Microbial Biochemistry, Bochum, Germany

Hypochlorous acid (HOCl) is a powerful oxidant produced in the phagolysosome of activated neutrophils. It rapidly reacts with all biomolecules of engulfed pathogens; however, proteins are considered to be a primary target. In proteins, HOCl most rapidly reacts with sulfur-containing amino acids, such as cysteine and methionine. Nevertheless, taking into consideration a higher abundance of amino groups over thiol groups, HOCl-induced modification of amines (*N*-chlorination) is frequently present in the cell. Here, we investigated a possible reduction mechanism for *N*-chlorinated proteins. The *E. coli* protein RidA turns into an effective chaperone-like holdase upon chlorination with HOCl (1). Interestingly, this chaperone activity of *N*-chlorinated RidA (RidA<sub>HOCl</sub>) can be reversed by the thiol-disulfide oxidoreductase TrxA, indicating a novel thioredoxin-induced reduction mechanism of *N*-chlorination. We generated the mutant TrxA<sub>C35S</sub>, which lacks the resolving cysteine and thus is not able to resolve mixed intermediates. Using catalytic amounts of TrxA and TrxA<sub>C35S</sub>, we demonstrated that the resolving cysteine C35 is required for the inactivation of RidA<sub>HOCl</sub>. Using a gel-shift assay, a product consisting of covalently-linked RidA-TrxA<sub>C35S</sub> was detected. We also demonstrated a covalently-linked product between TrxA and model chloramine. We propose a model of *N*-Cl reduction by thioredoxin, which involves the formation of an intermolecular sulfonamide between TrxA and its substrate. To identify the location of *N*-chlorination in RidA, we established a 5-(Dimethylamino) naphthalene-1-sulfinic acid (Dansyl sulfinic acid)-based probe. This probe can successfully label *N*-chloramines in RidA and we were able to gather direct evidence for *N*-chlorination of lysine KXXX.

1. Müller, A., Langklotz, S., Lupilova, N., Kuhlmann, K., Bandow, J.E., Leichert, L.I.O., 2014. Activation of RidA chaperone function by *N*-chlorination. *Nat. Commun.* 5, 5804.

## 273-MSRP

Analysis of TisB-dependent stress responses in *Escherichia coli*

\*N. E. Schmid<sup>1</sup>, D. Edelmann<sup>1</sup>, B. Berghoff<sup>1</sup>

<sup>1</sup>Justus-Liebig-Universität Gießen, Institut für Mikro- und Molekularbiologie, Gießen, Germany

Persister cells represent a small subpopulation that can be found in virtually all bacterial cultures. They are highly tolerant to stressors, including various antibiotics. Bacterial persistence is often linked to toxin-antitoxin systems. One of these systems is TisB/IstR-1 [1, 2]. The toxin TisB induces dormancy by causing depolarization of the cytoplasmic membrane and subsequent ATP depletion [3]. Expression of *tisB* is negatively controlled by the repressor LexA, the master regulator of the SOS response. Upon DNA damage LexA undergoes auto-cleavage, which results in strong *tisB* transcription and increasing amount of TisB protein. Hence, TisB-dependent persistence occurs during an activated SOS response. TisB insertion into the inner membrane might cause secondary stress. It was, e.g., observed that strong TisB expression leads to formation of reactive oxygen species and activation of the oxidative stress response [4]. Here, we analyzed TisB-dependent stress responses in *E. coli*. For this purpose, an inducible TisB expression system was constructed, which allows moderate overexpression of TisB and formation of a homogeneous TisB-dependent persister population. Induction of various stress responses was monitored at the transcriptional level using qRT-PCR. In addition, expression of individual stress response genes was validated using transcriptional *syf/p2*-fusions. The analysis

was also extended to the recovery phase after TisB overexpression. We show that the oxidative stress response, the heat shock response and the envelope stress response are triggered in a TisB-dependent manner. We speculate that these stress responses might also play a major role in TisB-dependent persistence.

References:

[1] Dörr *et al.* (2010) *PLoS Biol.* 8(2): e1000317.

[2] Berghoff *et al.* (2017) *Mol. Microbiol.* 103(6): 1020-1033.

[3] Unonso and Wagner (2008) *Mol. Microbiol.* 70(1):258-70.

[4] Edelmann and Berghoff (2019) *Sci Rep.* 9(1):14256.

## 274-MSRP

High sensitivity against membrane targeting antimicrobials in polyphosphate deficient *Pseudomonas aeruginosa*

\*S. Brückner<sup>1</sup>, W. Y. Wholey<sup>1</sup>, M. Hall<sup>2</sup>, U. Jakob<sup>1</sup>, J. U. Dahl<sup>2</sup>

<sup>1</sup>University of Michigan, Molecular, Cellular and Developmental Biology/Biology and Biotechnology, Ann Arbor (MI), United States

<sup>2</sup>Illinois State University, School of Biological Sciences, Normal (IL), United States

Infections by multi-drug resistant bacteria are a major threat to human health. Due to its intrinsic antibiotic resistance and ability to form strong biofilms, the opportunistic pathogen *Pseudomonas aeruginosa* is particularly difficult to treat and contributes i. a. to the progression of burn wounds. One promising approach to combat pathogens is to target bacteria-specific cellular processes that directly contribute to virulence and persistence and which become essential for bacterial survival only in the context of the host. One such candidate is polyphosphate kinase (PPK), a bacteria-specific enzyme that converts ATP into the universally conserved biopolymer polyphosphate (polyP) during various stress conditions to protect bacteria from stress-mediated protein aggregation and contribute to their survival. In addition, virulence is highly attenuated in polyP-deficient bacteria, as they are defective in biofilm formation and persistence. We recently found that colonization of porcine burn wounds by *P. aeruginosa* is 10 to 15-fold reduced if polyP production is compromised. Due to the many stress-related phenotypes known for *ppk*-deficient bacteria, we wondered whether clinically relevant antibiotics would sensitize polyP-deficient *P. aeruginosa* more efficiently than wild-type cells (WT) and thus could be used to reinforce the phenotype. Using the microdilution method, we compared the minimum inhibitory/bactericidal concentrations (MIC/MBC) in WT and *ppk*-deficient *P. aeruginosa* for numerous antibiotics that differ in their mode of action. No significant differences in MIC and MBC were observed for antibiotics targeting cell wall biosynthesis, translation, and DNA replication, respectively.

Intriguingly, we found that polyP-deficient cells were 100 to 100,000-fold more sensitive to different membrane-disrupting compounds including the cationic surfactant octenidine, polymyxin B, and the silver-containing antimicrobial surface-coating AgXX. Both silver and octenidine are commonly used in wound management. Future studies are necessary to determine whether the membrane-targeting compounds synergize the antimicrobial effects of the PPK-targeting drug mesalamine.

## 275-MSRP



Challenging antibiotic resistance – lipopolysaccharide synthesis as a potential target for an efficient treatment of Gram-negative bacteria

\*A. M. Möller<sup>1</sup>, S. Schäkermann<sup>1</sup>, N. Thomanek<sup>1</sup>, F. Narberhaus<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Microbiology and Biotechnology, Bochum, Germany

Today one of the biggest healthcare threats is the rapid emergence of antibiotic-resistant pathogens, causing an urgent need for new classes of antibiotics. Essential processes like DNA replication, protein synthesis or membrane and cell wall synthesis are suitable antimicrobial targets, illustrated by the revolutionary success of  $\beta$ -lactams. Contrary to most biological membranes, the outer membrane of many Gram-negative bacteria is unique due to its asymmetrical organization, with an inner leaflet of phospholipids and an outer leaflet that mainly contains lipopolysaccharides (LPS). Since many enzymes involved in LPS synthesis are essential, they are promising drug targets.

Among those, the key enzyme LpxC, which catalyzes the first committed step of LPS synthesis, emerged as potent inhibition target for a shutdown of LPS synthesis. Today, several inhibitors of LpxC are commercially available and tested *in vitro*. However so far, only little is known about the cellular response to those compounds. Our investigations focus on the proteomic response of *Escherichia coli* and other clinically relevant species like *Pseudomonas aeruginosa* upon exposure to LpxC inhibitors. Interestingly, the cell uses LpxC as a checkpoint for LPS synthesis. To maintain an optimal LPS level, LpxC is degraded in a growth-phase dependent manner by the membrane-anchored protease, FtsH. To date, the underlying regulatory mechanisms of the LpxC turnover are not well understood but are presumably controlled via direct protein-protein interactions. Proteins could either shield a degradation-motif or deliver LpxC actively to the protease. We want to validate direct LpxC interaction partners by analyzing promising candidates with the bacterial-two hybrid approach *in vivo* as well as *in vitro* using pull-down assays. Both projects attempt to pave the way for the development of a new class of antibiotics, to counteract rapidly evolving multi-resistant strains.

---

## 276-MSRP

### CRISPR-Cas Systems in *Rhodobacter capsulatus*

\*J. Kretz<sup>1</sup>, D. T. Spanka<sup>1</sup>, G. Klug<sup>1</sup>

<sup>1</sup>Institut für Mikro- und Molekularbiologie, Gießen, Germany

CRISPR-Cas systems are mostly seen as a kind of adaptive immune system in archaea and bacteria. Genomic fragments of invading viruses are integrated into the CRISPR locus and prevent, together with Cas-proteins, further infection of the same virus by degrading the viral genome. Over the last years CRISPR-Cas systems were investigated in other directions than just being an adaptive immune system. CRISPR-Cas systems were found to participate in internal gene regulation and regulation of developmental programs, as well as stress response in different bacteria. Mainly membrane stress was found to activate CRISPR-Cas systems, which also occurs during the attachment of viruses. This project targets the CRISPR-Cas systems of the alphaproteobacterium *Rhodobacter capsulatus* and elucidates whether it is functional beyond adaptive immunity. Three different CRISPR-Cas systems were found through RNA-Seq data comparing non stressed with photooxidative stressed cultures, indicating a function in stress response to photooxidative stress. Besides from the indication of participation in photooxidative stress response, different CRISPR-RNAs were shown to strongly depend on growth phase. Some CRISPR-RNAs are especially active in late

stationary phase and outgrowth. In order to investigate the function of the CRISPR-Cas systems, different stressors will be applied and after that RNA will be extracted and analysed via Northern Blot Analysis. Aside from the different stressors the activity of CRISPR-RNAs will be monitored in different growth phases. Furthermore, different Cas proteins will be removed and overexpressed in order to investigate the function of the proteins in *R. capsulatus*. Especially the Cas13a protein will be investigated, which cannot be knocked out. To investigate this protein, an IPTG inducible construct will be used in order to deplete the protein. Besides that, all proteins will be tested for function in the closely related bacterium *Rhodobacter sphaeroides*, which does not contain any CRISPR-Cas systems.

---

## 277-MSRP

### Cyanobacterial acclimation to carbon limitation reveals complex regulation on proteome and phosphoproteome levels.

\*P. Spät<sup>1</sup>, T. Barske<sup>2</sup>, B. Maček<sup>1</sup>, M. Hagemann<sup>2</sup>

<sup>1</sup>University Tuebingen, Quantitative Proteomics, Tübingen, Germany

<sup>2</sup>University of Rostock, Department of Plant Physiology, Rostock, Germany

Cyanobacteria such as *Synechocystis* sp. PCC 6803 are phototrophic prokaryotes, which depend on a balanced carbon-to-nitrogen supply to maintain full metabolic capacity. Fluctuating supply of inorganic carbon in aquatic environments requires a fine regulation of the cyanobacterial inorganic carbon concentrating mechanisms (CCM), involving diverse CO<sub>2</sub> uptake systems and carboxysome proteins, as well as many associated processes. To reveal which levels of cellular regulation are involved in the acclimation to inorganic carbon limitation, we performed a quantitative phosphoproteome study, applying high-accuracy mass spectrometry. By comparing cells from carbon saturated conditions (5% CO<sub>2</sub>) with cells from short (3 hours) and long term (24 hours) carbon limitation conditions (ambient air with 0.04% CO<sub>2</sub>), we obtained a comprehensive and reproducible dataset. Overall, we achieved an in-depth proteome coverage, identifying 66% of all protein coding genes, with quantitative information for more than 2200 proteins between the different conditions. Furthermore, the profiles of about 120 O-phosphorylation events could be analyzed. As expected and validated by previous transcriptome studies, CO<sub>2</sub> and bicarbonate uptake associated proteins were significantly upregulated, whereas the expression of proteins involved in nitrogen import and assimilation was inhibited. In contrast to overall congruent transcriptome and proteome dynamics, also distinct differences could be analyzed, which clearly point at a layer of yet not understood post-transcriptional regulation. Interestingly, we could identify subunits of the bicarbonate transporter complex to be modified by O-phosphorylation, which revealed a complex change in their abundance and phosphorylation level, when carbon supply is limited. This suggests that O-phosphorylation represents an additional level of regulation for the maintenance of the cellular carbon-to-nitrogen balance.

---

## 278-MSRP

Quantitative proteome profiling of *Coxiella burnetii* reveals major metabolic and stress differences under axenic and cell culture cultivation.

\*K. Mertens-Scholz<sup>1</sup>, J. Dresler<sup>2</sup>, P. Pajer<sup>2</sup>, H. Neubauer<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institut für bakterielle Infektionen und Zoonosen, Jena, Germany

<sup>2</sup>Military Health Institute, Prague, Czech Republic



*Coxiella burnetii*, an obligate intracellular bacterium, is the causative agent of the zoonosis Q fever. Classical genetic approaches are not routinely used and transcriptomic data often-missing confirmation on the proteome level. The aim is to use a semi-quantitative proteomics approach to identify isolate specific traits. *C. burnetii* phase I (NMI) and phase II (NMII) were compared during growth in axenic media with persistently infected mouse fibroblasts. Overall, 659 and 1046 *C. burnetii* proteins of 2132 annotated coding sequences were identified. Proteome profiles clustered accordingly to the culturing conditions used and indicated different regulation patterns. NMI proteome profiles in axenic media indicate transition from exponential to stationary phase. Regulators such as RpoS, CsrA2, UspA1 and UspA2 were upregulated. Additionally, upregulation of several cell envelope processing, cell division proteins indicate transition from large cell variant to small cell variant. Translocation of proteins via the Sec translocon or type IV B secretion system is present under all conditions. Upregulation of oxidative stress response and transporters associated with osmoregulation was shown. This is the first semi-quantitative analysis of axenically grown with cell culture propagated *C. burnetii* at the proteomic level. Particularly, transition from exponential to stationary phase and likely adaptations in response to the environment of the parasitophorous vacuole was demonstrated.

---

## 279-MSRP

### Role of the stringent response for biofilm formation in *Staphylococcus aureus*

\*A. Salzer<sup>1</sup>, D. Keinhorster<sup>1</sup>, C. Kästle<sup>1</sup>, B. Kästle<sup>1</sup>, C. Wolz<sup>1</sup>

<sup>1</sup>University of Tübingen, Interfaculty Institute for Microbiology and Infection Medicine, Tübingen, Germany

In *Staphylococcus aureus* the stringent response is activated by either amino acid limitation or cell wall stress. Subsequently, the alarmones guanosine tetra- and pentaphosphate, namely (p)ppGpp, are produced by the RelA/SpoT homologue (RSH), that is activated upon amino acid starvation, and the two small alarmone synthases RelP and RelQ that are induced under cell wall stress. The stringent response was shown to impact biofilm formation in different organisms and anti-biofilm peptides were proposed to block (p)ppGpp, as important signal in biofilm development. Here, the influence of the stringent response on biofilm formation in different *S. aureus* mutant strains lacking one, two or all (p)ppGpp synthases was analysed in a microtiter plate biofilm assay. Under relaxed conditions, no significant differences between mutants and wild type were detectable. Addition of sub-inhibitory concentrations of cell wall active vancomycin significantly reduced biofilm formation in mutants lacking *relP* and *relQ* when compared to (p)ppGpp producing parental strain. Treatment of the biofilm with DNase, proteinase or NaIO<sub>4</sub> revealed that the biofilm matrix is mostly composed of proteins and eDNA. The proposed (p)ppGpp inhibitory peptides DJK-5 and IDR-1018 showed biofilm inhibiting activity in wild type and mutant strains indicating that in *S. aureus* these anti-biofilm molecules act independently of (p)ppGpp. In conclusion, (p)ppGpp produced by RelP and RelQ contributes to the maintenance of biofilm under cell wall stress conditions.

---

## 280-MSRP

### The tripartite efflux pump TtgABC confers resistance to iron chelators and other toxic compounds

\*N. V. Stein<sup>1</sup>, T. Henriquez<sup>1</sup>, T. Baldow<sup>1</sup>, H. Jung<sup>1</sup>

<sup>1</sup>Ludwig-Maximilians-Universität München, Mikrobiologie, Martinsried, Germany

*Pseudomonas putida* KT2440 is a Gram-negative, rod shaped bacterium that colonizes the roots of plants and soil and is part of freshwater communities. In this context, *P. putida* is adapted to cope with toxic environmental conditions and tripartite efflux pumps, such as TtgABC (homolog of MexAB-OprN in *Pseudomonas aeruginosa*), belong to the main molecular players in this process. Previous publications revealed a role of TtgABC of *P. putida* in the secretion of toluene and beta lactams. However, the involvement of this efflux system in the transport of other compounds remains to be clarified. Therefore, in order to get a better understanding of its biological function, the aim of this work was to identify novel substrates of TtgABC of *P. putida*.

To that end, a mutant strain with a deletion for the gene encoding the inner membrane component of the system (*ttgB*) was generated and characterized. In parallel, the complementation of *ttgB* was assessed through the use of a low copy number plasmid. All strains were phenotypically characterized (growth curves in different conditions) and susceptibility tests for antibiotics through disc diffusion method and minimum inhibitory concentration (using strips) were performed. Additional tests, such as fluorescent microscopy, were performed to characterize specific phenotypes.

Our results revealed that TtgABC possesses a broad substrate specificity for compounds of both mutagenic and toxic nature. These compounds are ranging from dyes like ethidium bromide to metabolites like deoxycholic acid. In addition, one widely used iron-chelator, 2'2'-bipyridyl, is extruded by this system. Additionally, different classes of antibiotics showed decreased minimum inhibitory concentrations if tested on the mutant strains.

These results indicate that a wide substrate specificity represents a conducive survival strategy of *Pseudomonas putida* KT2440. Due to the broad range of compounds transported by TtgABC, a fast adaption into new environmental circumstances is possible.

---

## 281-MSRP

### The impact of a novel ClpA mutation on MazEF antitoxin-toxin system in *Escherichia coli* in the presence of antibiotic stress.

\*A. Schwarz<sup>1</sup>, A. Heisig<sup>1</sup>, P. Heisig<sup>1</sup>

<sup>1</sup>Universität Hamburg / Institut für Biochemie und Molekularbiologie, Pharmazeutische Biologie und Mikrobiologie, Hamburg, Germany

## Question

ClpA is the ATP-binding subunit of the ATP-dependent protease complex ClpAP, which mediates the degradation of misfolded and some specific labile proteins. Mutations in *clpA* have been demonstrated to prevent the formation of this protease complex. Consequently, beside affecting the general protein turn-over, the degradation of specific target proteins such as MazE is affected. MazE is the labile antitoxin component of the MazEF antitoxin-toxin system in *Escherichia coli*.

The aims of this study were to investigate (1) the impact of *clpA* mutations on the activity of the MazEF antitoxin-toxin system and (2) the ability of *clpA* mutants to survive after exposition to antibiotic stress.

## Methods

The mutation ClpA-Ala569Argfs\*12 was introduced into the genome of an *E. coli* wild-type strain by using CRISPR/Cas9 (no-SCAR method of Reisch&Prather).

Susceptibility testing followed EUCAST protocol for broth dilution, but using linear dilution steps.

For the growth experiments the wild-type strain and the isogenic *clpA* mutant were grown to early exponential phase followed by induction with 1x MIC of the respective antibiotic. The growth was measured with the live-cell imaging system oCelloScope™.

## Results

For both strains (wild-type and mutant) susceptibility and the growth curves in the absence of antibiotic generated with the oCelloScope™ were identical. However, in the presence of e.g. rifampicin (1x MIC) the *clpA* mutant strain showed a faster growth compared to the wild-type.

## Conclusions

The survival rate of the *clpA* mutant strain after induction by rifampicin was higher than that of the wild-type. This may be explained by a probably higher amount of MazE due to the diminished degradation. As a consequence the ratio of antitoxin to toxin is shifted.

## References

Reisch CR, Prather KL. The no-SCAR (Scarless Cas9 Assisted Recombineering) system for genome editing in *Escherichia coli*. *Sci Rep*. 2015;5:15096. Published 2015 Oct 14. doi:10.1038/srep15096

### 282-MSRP

The cellular interplay of zinc homeostasis and folate metabolism in *C. metallidurans*

\*V. Schulz<sup>1</sup>, M. Herzberg<sup>1</sup>, D. H. Nies<sup>1</sup>

<sup>1</sup>Martin Luther- University of Halle-Wittenberg, Molecular Microbiology, Halle, Germany

**Introduction:** Zinc as one of the biometals is an important trace element and about 5% of all proteins in prokaryotes are zinc-binding proteins, even though zinc is toxic in high concentration. Folate biosynthesis is an essential metabolic pathway that starts with the conversion of guanosine-5'-triphosphate (GTP) into 7,8 dihydroneopterin triphosphate (7,8 DHNTP). This metabolite leads via branched pathways to modified RNA nucleosides, tetrahydroneopterin and folic acid biosynthesis. The importance of zinc in the folate metabolism is reflected by the first step of the de novo biosynthesis, which is catalyzed by a zinc dependent enzyme, the GTP cyclohydrolase I (FolE<sub>1A</sub>). In contrast to the most common model organism *E. coli*, *C. metallidurans* possess two further enzymes of another subfamily – FolE<sub>1B1</sub> and FolE<sub>1B2</sub>. Both of the paralogues are predicted to be cambialistic with ability to use different cofactors than Zn<sup>2+</sup>-like Fe<sup>2+</sup> or Co<sup>2+</sup> to maintenance enzyme activity.

**Objectives:** The main aim of our studies is to investigate, how a disturbed zinc and general metal homeostasis affects the folate metabolism. To underline are the role of the putative zinc chaperone CobW<sub>1</sub>, the interplay of the three FolE-paralogues and the biochemical studies of those three enzymes.

**Material & Methods:** Protein purification, enzyme activity assays and cofactor determination for biochemical characterization, reporter gene assays to determine the induction condition and creation as well as physiological characterization of deletion mutants.

**Results:** Whereas *folE<sub>1B1</sub>* is constitutively expressed, the expression of *folE<sub>1B2</sub>* is dependent on the zinc status in the cell and controlled by the Zinc uptake regulator Zur. Together with the *cobW<sub>1</sub>*-gene upstream and four further genes downstream, it forms a Zur-regulated gene cluster. As a part of a Zur-regulon, it is involved in the maintenance of the zinc homeostasis. The FolE<sub>1B</sub> proteins show enzyme activity with different cofactors.

**Conclusion:** Zur-regulated but zinc-independent enzymes, such as FolE<sub>1B2</sub>, facilitates this model organism the survival in zinc deficiency environments through the use of alternative metal-cofactors in cambialistic enzymes.

### 283-MSRP

Transcriptomic and proteomic profiling of the *Aspergillus nidulans* response to low temperature stress revealed a distinct profile of secondary metabolites and the induction of sexual development

B. Hanf<sup>1</sup>, T. Krüger<sup>1</sup>, D. J. Mattern<sup>1</sup>, M. Stroe<sup>1</sup>, T. Conrad<sup>2</sup>, B. Bunk<sup>3</sup>, A. M. Köhler<sup>4</sup>, A. Strohdiek<sup>4</sup>, G. Braus<sup>4</sup>, J. Overmann<sup>3</sup>, A. A. Brakhage<sup>1</sup>, \*O. Kniemeyer<sup>1</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology (HKI), Molecular and Applied Microbiology, Jena, Germany

<sup>2</sup>Leibniz Institute for Natural Product Research and Infection Biology (HKI), System Biology and Bioinformatics, Jena, Germany

<sup>3</sup>Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

<sup>4</sup>Georg-August-University Göttingen, Institute for Microbiology and Genetics, Department of Molecular Microbiology and Genetics, Göttingen, Germany

Filamentous fungi are ubiquitous eukaryotic microorganisms found in the environment in association with soil, plants, animals or bacteria. The seasonal cycle exposes fungi to regular temperature changes. For tolerating low temperatures, a cellular cold stress response is essential. However, little is known about how filamentous fungi cope with low temperature-induced stress. To gain detailed insights into the low temperature adaptation of the important model fungus *Aspergillus nidulans*, we analyzed the dynamics of the transcriptome, proteome, and secondary metabolome during low temperatures that still allow growth. Our multi-omics approach revealed that genes and proteins involved in the categories cold stress protection, cell development and biosynthesis of natural products were enriched at 10°C. Common cold protective mechanisms, such as osmotic stress response proteins and DNA repair enzymes, were induced. In addition, an altered secondary metabolite (SM) production profile was confirmed at the metabolite level by LC-MS/MS analysis. Sharp temperature shifts triggered the expression of silent gene clusters such as known SMs, e.g. asperfuranone and the anthraquinone pigment asperthecin, as well as so-far uncharacterized SMs. Noteworthy, cold- induced SMs extracted from culture supernatants inhibited growth of Gram-positive bacteria and fungi. Furthermore, our analyses revealed the induction of factors, which regulate the sexual cycle in *A. nidulans*. A shift towards sexual development was confirmed by the observation that Hülle cells were formed at 10°C in liquid cultures. We are now analyzing *A. nidulans* mutants defective in sexual development to provide more insights into the underlying regulatory mechanisms of cold adaptation and Hülle cell formation.

---

## 284-MSRP

### Regulatory Network of Bce-like Modules in *Lactobacillus casei* BL23 in Response to Subtilin

\*Q. Zhang<sup>1</sup>, A. Revilla-Guarinos<sup>1</sup>, C. Alcántara<sup>2</sup>, T. Mascher<sup>1</sup>, M. Zúñiga<sup>2</sup>, D. Wolf<sup>1</sup>

<sup>1</sup>Technische Universität(TU) Dresden, Department of Microbiology, Dresden, Germany

<sup>2</sup>Consejo Superior de Investigaciones Científicas(CSIC), Valencia, Spain

In *Firmicutes*, sensory complexes comprised of an ATP-binding cassette (ABC) transporter and a two-component system (TCS) have been recognized as important antimicrobial peptide (AMP) resistance modules. One paradigm example for such a module represents the BceRS-BceAB system in *Bacillus subtilis*[1]. In *L. casei* BL23, the Bce-like modules, ApsRSAB and PsdRSAB as well as an orphan ABC transporter DerAB, have been identified for their ability to confer resistance against AMPs[2]. This study aims to investigate the regulatory network of these Bce-like modules responding to lantibiotic subtilin in *L. casei*. Results from minimal inhibitory concentration (MIC) assays and preliminary qRT-PCR showed that the PsdRSAB system possesses the characteristic features of a Bce-like module with PsdAB perceiving and detoxifying subtilin mediated by PsdRS. Surprisingly, a unique resistance pathway was observed via ApsRSAB and DerAB modules. Here, instead of the typical dual-function of BceAB-like ABC transporter, ApsAB only acts as a sensor that activates TCS ApsRS to induce expression of the DerAB transporter, which in turn mediates the actual resistance (ApsRS-ApsAB-DerAB). This unique regulatory module of a TCS associated with two ABC transporters playing separate roles in signal transduction and antibiotic resistance have been reported in other *Firmicutes*[3,4,5]. Our findings further emphasize the widespread occurrence and conservation of this unique circuit. Moreover, the *dlt* operon (for teichoic acid synthesis) regulated by ApsR was identified as a significant resistance determinant for the cationic AMP subtilin, as well as the *mprF* gene (for phospholipids synthesis) but to a lesser degree. In summary, our results demonstrate a subtilin resistance network in *L. casei* formed by a typical Bce-like module, PsdRS-PsdAB, and a unique multicomponent module ApsRS-ApsAB-DerAB.

[1] Ohki et al. (2003) Mol Microbiol. 49(4):1135-44.

[2] Revilla-Guarinos et al. (2013) Appl Environ Microbiol. 79(10):3160-70.

[3] Hiron et al. (2011) Mol Microbiol. 81(3):602-22.

[4] Jiang et al. (2019) Appl Environ Microbiol. 85:e01470-19.

[5] Gebhard et al. (2014) Antimicrob Agents Chemother. 58(3):1425-33.

---

## 285-MSRP

The author has not agreed to a publication.

---

## 286-MSRP

### The impact of Surfactin on protein physiology of *B. subtilis*

\*L. Lilge<sup>1</sup>, M. Vahidinasab<sup>1</sup>, J. Pfannstiel<sup>1</sup>, B. Würtz<sup>1</sup>, M. Henkel<sup>1</sup>, R. Hausmann<sup>1</sup>

<sup>1</sup>Food Science and Biotechnology, Stuttgart, Germany

The cyclic lipopeptide Surfactin is described as a biosurfactant with antibacterial characteristics. In the case of *B. subtilis* 168, the Surfactin synthetase-activating enzyme *sfp* is inactive (*sfp*<sup>-</sup>). Consequently, cell growth proceeds without Surfactin production. By repairing this pseudogene (*sfp*<sup>+</sup>), lipopeptide production is restored.

Accordingly, the impact of Surfactin on cell physiology could be analyzed by comparing protein compositions and quantities in both strains (*sfp*<sup>+</sup> and *sfp*<sup>-</sup>). To get an idea about the cellular adaptations during the cultivation process, multiple time points from exponential as well as stationary phase were investigated. The protein expression profiles were determined by mass spectrometry resulting in a promising list of proteins, which exhibited remarkable differences between these strains.

Apart from different transporters, regulatory systems for iron homeostasis, sporulation and biofilm formation were identified. In this way, several bacterial strategies could be revealed to deal with Surfactin accumulation in physiological concentrations. Based on these insights, novel targets were conceived for strain engineering to obtain an optimized cell growth for best possible Surfactin production.

---

## 287-ZOP

### Murine fecal microbiota transplantation alleviates intestinal and systemic immune responses in *Campylobacter jejuni* infected mice harboring a human gut microbiota

\*M. M. Heimesaat<sup>1</sup>, K. Mrazek<sup>1</sup>, S. Bereswill<sup>1</sup>

<sup>1</sup>Charité - University Medicine Berlin, Institute of Microbiology, Infectious Diseases and Immunology, Berlin, Germany

Human campylobacteriosis constitutes an emerging zoonotic food-borne disease of significant socioeconomic impact. We have recently shown that conventional mice are protected from *Campylobacter jejuni* infection, which was not the case for human microbiota associated (hma) mice indicating that the host-specific gut microbiota composition primarily determines susceptibility towards or resistance against *C. jejuni* infection. In our present preclinical intervention study we addressed whether gut microbiota changes in stably *C. jejuni* infected hma mice following murine fecal microbiota transplantation (mFMT) could alleviate pathogen-induced immune responses. Therefore, secondary abiotic C57BL/6 mice were generated by broad-spectrum antibiotic treatment, perorally reassociated with a complex human gut microbiota and challenged with *C. jejuni* by gavage. Seven days later *C. jejuni* infected hma mice were subjected to peroral mFMT on three consecutive days. Within a week post mFMT fecal pathogenic burdens had decreased by two orders of magnitude, whereas distinct changes in the gut microbiota composition with elevated numbers of lactobacilli and bifidobacteria could be assessed. In addition, mFMT resulted in less *C. jejuni* induced apoptotic responses in colonic epithelia, reduced numbers of macrophages and monocytes as well as of T lymphocytes in the large intestinal mucosa and lamina propria and in less distinct intestinal pro-inflammatory cytokine secretion as compared to mock challenge. Strikingly, inflammation-dampening effects of mFMT were not restricted to the intestinal tract, but could also be observed systemically. In conclusion, our preclinical mFMT intervention study provides evidence that changes in the gut microbiota composition which might be achieved by pre- or probiotic formulations may effectively lower intestinal *C. jejuni* loads, dampen both, pathogen-induced intestinal and systemic inflammatory sequelae and may represent a useful tool to treat continuous shedding of *C. jejuni* by asymptomatic carriers which is critical in the context of food production, hospitalization and immunosuppression.

## 288-ZOP

Prevalence and antimicrobial susceptibility of *Arcobacter* species in human stool samples: the prospective German *Arcobacter* prevalence study Arcopath

\*M. M. Heimesaat<sup>1</sup>, V. Brückner<sup>2</sup>, U. Fiebiger<sup>1</sup>, R. Ignatius<sup>3</sup>, J. Friesen<sup>3</sup>, M. Höck<sup>4</sup>, M. Eisenblätter<sup>5</sup>, T. Alter<sup>2</sup>, S. Bereswill<sup>1</sup>, G. Götz<sup>2</sup>

<sup>1</sup>Charité - University Medicine Berlin, Institute of Microbiology,

Infectious Diseases and Immunology, Berlin, Germany

<sup>2</sup>Freie Universität Berlin, Institute of Food Hygiene, Berlin, Germany

<sup>3</sup>Labor 28, Berlin, Berlin, Germany

<sup>4</sup>Labor Limbach, Berlin, Berlin, Germany

<sup>5</sup>Synlab MVZ, Berlin, Berlin, Germany

*Arcobacter* species, particularly *A. butzleri*, but also *A. cryaerophilus* constitute emerging pathogens causing gastroenteritis in humans. However, isolation of *Arcobacter* may often fail during routine diagnostic procedures due to the lack of standard protocols. Furthermore, defined breakpoints for the interpretation of antimicrobial susceptibilities of *Arcobacter* are missing. Hence, reliable epidemiological data of human *Arcobacter* infections are scarce and also lacking for Germany. We therefore performed the 13-month prospective *Arcobacter* prevalence study Arcopath in German patients. A total of 4646 human stool samples was included and *Arcobacter* spp. were detected in 0.85% of specimens from 3884 outpatients and in 0.40% of samples from 752 hospitalized patients. Whereas *A. butzleri*, *A. cryaerophilus* and *A. lanthieri* were identified in outpatients, only *A. butzleri* could be isolated from samples of hospitalized patients. Overall, *A. butzleri* was the most prevalent species ( $n = 24$ ; 67%), followed by *A. cryaerophilus* ( $n = 10$ ; 28%) and *A. lanthieri* ( $n = 2$ ; 6%). Antimicrobial susceptibility testing of *Arcobacter* isolates revealed high susceptibilities to ciprofloxacin, whereas bimodal distributions of MICs were observed for azithromycin and ampicillin. In summary, *Arcobacter* could be isolated in 0.85% of German outpatients and ciprofloxacin rather than other antibiotics might be appropriate for antibiotic treatment of infections. Further epidemiological studies are needed, however, to provide a sufficient risk assessment of *Arcobacter* infections in humans.

## 289-ZOP

Vitamin D in acute campylobacteriosis – results from an intervention study applying a clinical *Campylobacter jejuni* induced enterocolitis model

\*S. Bereswill<sup>1</sup>, S. Mousavi<sup>1</sup>, F. D. Lobo de Sá<sup>2</sup>, J. Schulzke<sup>2</sup>, R. Bückner<sup>2</sup>, M. M. Heimesaat<sup>1</sup>

<sup>1</sup>Charité - University Medicine Berlin, Pharmaceutical Microbiology Group/ Microbiology, Infectious Diseases and Immunology, Berlin, Germany

<sup>2</sup>Charité - University Medicine Berlin, Institute of Clinical Physiology, Berlin, Germany

Human *Campylobacter* infections are progressively rising and of high socioeconomic impact. In the present preclinical intervention study we investigated anti-pathogenic, immunomodulatory and intestinal epithelial barrier preserving properties of vitamin D applying an acute campylobacteriosis model. Therefore, secondary abiotic IL-10<sup>-/-</sup> mice were perorally treated with synthetic 25-OH-cholecalciferol starting four days before peroral *Campylobacter jejuni* infection. Whereas 25-OH-cholecalciferol application did not affect gastrointestinal pathogen loads, 25-OH-cholecalciferol treated mice suffered less frequently from diarrhea in the midst of infection as compared to placebo control mice. Moreover, 25-OH-cholecalciferol application dampened *C. jejuni* induced apoptotic cell responses in colonic epithelia and promoted cell-regenerative measures. At day 6 post-infection, 25-OH-cholecalciferol treated mice displayed lower

numbers of colonic innate and adaptive immune cell populations as compared to placebo controls that were accompanied by lower intestinal concentrations of pro-inflammatory mediators including IL-6, MCP1 and IFN- $\gamma$ . Remarkably, as compared to placebo application synthetic 25-OH-cholecalciferol treatment of *C. jejuni* infected mice resulted in lower cumulative translocation rates of viable pathogens from the inflamed intestines to extra-intestinal including systemic compartments such as the kidneys and spleen, respectively, which was accompanied by less compromised colonic epithelial barrier function in the 25-OH-cholecalciferol as compared to the placebo cohort. In conclusion, our preclinical intervention study provides evidence that peroral synthetic 25-OH-cholecalciferol application exerts inflammation-dampening effects during acute campylobacteriosis.

## 290-ZOP

Pathogenicity and virulence of *S. schweitzeri* – a new member of the *S. aureus* complex

\*A. Großmann<sup>1</sup>, N. Froböse<sup>1</sup>, A. S. Alabi<sup>2</sup>, A. Mellmann<sup>3</sup>, F. Schaumburg<sup>1</sup>, S. Niemann<sup>1</sup>

<sup>1</sup>Westfälische Wilhelms-Universität Münster, Institute for Medical Microbiology, Münster, Germany

<sup>2</sup>Centre de Recherches Médicales de Lambaréné, Lambaréné, Gabon

<sup>3</sup>Westfälische Wilhelms-Universität Münster, Institute for Hygiene, Münster, Germany

## Introduction

*Staphylococcus schweitzeri* belongs to the *Staphylococcus aureus* complex and is mostly found in Africa wildlife so far. The capacity of *S. schweitzeri* to cause disease in humans remains controversial since no clinical infection has been reported, yet.

## Objectives

The aims of this work were to test the virulence of *S. schweitzeri* in a wide range of *in vitro* assays on the largest *S. schweitzeri* collection published so far and to screen systematically clinical samples for *S. schweitzeri*.

## Materials & methods

Whole genome sequencing was done to construct a phylogenetic tree of *S. schweitzeri* ( $n=58$ ) from Gabon, DR Congo, Nigeria and Côte d'Ivoire. The capacity for invasion, intracellular cell death induction, cytotoxicity, phagolysosomal escape, coagulase activity, biofilm formation and host cell activation of *S. schweitzeri* was compared with *S. aureus* isolates representing the most common clonal complexes (CC) in Africa (CC15, CC121, CC152) using different host cells (Vero, A549). A collection of clinical isolates from Gabon provisionally identified as *S. aureus* ( $n=159$ ) were screened for the presence of *S. schweitzeri*.

## Results

The population of *S. schweitzeri* comprises five geographical clusters and isolates from humans are found in two different clades within the Gabonese cluster. The comparison of *S. schweitzeri* vs. *S. aureus* comparator isolates showed a similar host cell invasion (0.9 vs. 1.2 CFU/ml, Vero), host cell activation (4.1 vs. 1.7 normalized fold expression of CCL5 and 7.3 vs. 9.9 normalized fold expression of IL8) and intracellular cytotoxicity (31.5% vs. 25%, A549). The extracellular pathogenicity (52.9% vs. 28.8%) was higher for

*S. schweitzeri* than for *S. aureus*. All tested *S. schweitzeri* were able to escape from phagolysosomes. *S. schweitzeri* was not detected in clinical samples.

## Conclusion

*S. schweitzeri* is as virulent as *S. aureus* in the applied in-vitro assays. Its transmission to humans was demonstrated on two occasions but clinical infections were not detected. *S. schweitzeri* might become an emerging pathogen in humans, if a cross-species transmission from African wildlife sustainably occurs.

## 291-ZOP

Single cell assay for DNA uptake in *Campylobacter jejuni* - a tool for monitoring the adaptive potential of the food-borne pathogen

\*J. Golz<sup>1</sup>, K. Stingl<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Berlin, Germany

## Introduction

*Campylobacter jejuni* is the major cause of bacterial induced gastroenteritis in the developed countries. The adaptive potential of *C. jejuni* is very high. Natural transformation can increase genetic diversity and, therefore, the adaptive potential.

## Objectives

In order to develop reduction strategies, it is important to understand the mechanisms of competence development and DNA uptake in *C. jejuni*. Therefore we developed a single cell based assay to monitor DNA-uptake.

## Materials and methods

To visualize DNA uptake *C. jejuni* was incubated with fluorescently labelled DNA and analyzed on a single-cell level. The ratio of competent cells was calculated. We genetically constructed mutants lacking homologues of either *pilQ*, *comE* or *comEC*, which were shown to be essential for DNA uptake in other bacteria.

## Results

Using the established single cell assay we visualized DNA uptake in *C. jejuni* and analyzed the competence development. Approximately 20 % to 30 % of *C. jejuni* cells took up fluorescently labelled *C. jejuni* DNA under standard conditions and showed DNase resistant fluorescent foci. The  $\Delta pilQ$  mutant was deficient for DNA uptake, as expected from the predicted role as outer membrane DNA channel in *Neisseria*. The  $\Delta comE$  and  $\Delta comEC$  mutants showed wild-type levels of DNA uptake (into the periplasm). Although ComE is known as a DNA-binding protein facilitating DNA uptake, our results indicate no essential role in DNA uptake in *C. jejuni*. Parallel transformation experiments with the  $\Delta comEC$  mutant showed no transformation activity. This is in accordance with ComEC being the inner membrane channel.

## Conclusions

The developed single cell DNA uptake assay provides visualization of DNA uptake. Two mutants showed defects in natural transformation. These mutants will be further analyzed, in cooperation with members of the PAC-CAMPY

consortium, for their adaptive potential *in vivo* and their ability to form biofilms.

## 292-ZOP

Genetic diversity of *Mycobacterium bovis* and its zoonotic potential in Ethiopia: A systematic review

\*B. Tulu<sup>1</sup>, G. Ameni<sup>1</sup>

<sup>1</sup>Bahir Dar University, Medical Laboratory Sciences, Bahir Dar, Ethiopia

**Background:** Understanding the types of *Mycobacterium bovis* strains circulating in a country and exploring its zoonotic potential has significant contribution in the effort to design control program. The main aim of this study was to review and compile the results of studies conducted on strains of *M. bovis* from animal and human in Ethiopia.

**Methods:** A systematic search and review of articles published on *M. bovis* strains in Ethiopia were made. PubMed and Google Scholar databases were considered for the search while the keywords used were "Mycobacteria," "*Mycobacterium bovis*," "Bovine Tuberculosis" and "Ethiopia."

**Result:** Fourteen studies were considered in this review and a total of 31 distinct strains of *M. bovis* (N=211) were obtained; the most dominant strains were SB0133 (N=62, 29.4%), SB1176 (N=61, 28.9%), and followed by SB0134 and SB1476 each (N=18, 8.5%). Regarding the clustering rate, 42.0% of the similar strains of *M. bovis* were found to be reported more than ones. On the other hand, 6 distinct strains of *M. bovis* (N=11, 5.2%) were reported from human subjects namely; SB0665 (N=4), SB0303 (N=2), SB0982 (N=2), SB0133 (N=1), SB1176 (N=1), and 1 new strain.

**Conclusion:** The result showed that the genetic diversity of *M. bovis* strains reported from Ethiopia are less diversified and highly clustered. And also the result underlines that there is an ongoing active transmission of *M. bovis* between human and animals in Ethiopia because a significant number of strains were reported from human and animals.

**Keywords:** *M. bovis*, zoonotic TB, *M. tuberculosis*, genetic diversity, Ethiopia

## 293-ZOP

Whole genome sequencing of methicillin-resistant staphylococci from German dairy farms revealed broad antimicrobial resistance profiles but a low risk of human infections

\*T. Lienen<sup>1</sup>, A. Schnitt<sup>1</sup>, S. Maurischat<sup>1</sup>, B. A. Tenhagen<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Berlin, Germany

**Introduction:** Methicillin-resistant *Staphylococcus aureus* (MRSA) can be a serious problem in dairy farming. Infections caused by MRSA cannot be treated with beta-lactam antibiotics that are the first line treatment for bovine mastitis. Beside MRSA, methicillin-resistant coagulase negative staphylococci (MR-CoNS) are frequently found on dairy farms. Considering the one health approach, MRSA and MR-CoNS might be transmitted from animals to humans and vice versa.

**Objectives:** The interest of the study is to analyze the genotypes, virulence factors and antimicrobial resistance gene profiles of MRSA and MR-CoNS from German dairy farms.

**Materials and Methods:** On preselected dairy farms with a history of MRSA detection, quarter milk, bulk tank milk and swab samples of calves, heifers and from the environment were analyzed for methicillin-resistant staphylococci. A selection of isolates was analyzed using whole genome sequencing.

**Results:** MRSA and MR-CoNS were detected in different sample types across the dairy farms. The methicillin-resistance was associated with the *mecA* gene in SCC*mec* types I, IVa, IVc, IVd and V as well as unknown types in various MR-CoNS isolates. Interestingly, phenotypically resistant but *mecA*- and *mecC*-negative *Staphylococcus cohnii* isolates from different farms showed modified penicillin-binding-protein genes. The detected MRSA strains were attributed to the livestock-associated clonal complex 398. Resistances to a broad range of antimicrobials such as aminoglycosides, beta-lactams, macrolides and tetracycline were predicted. However, the isolates lacked typical human infection associated genes such as the immune evasion cluster genes *sak*, *chp*, *scn*, staphylococcal enterotoxin genes or Panton-Valentine leukocidin genes.

**Conclusion:** MRSA and MR-CoNS are widely spread across the dairy farm environment carrying a broad variety of antimicrobial resistance genes. It cannot be ruled out that the resistance genes can be transmitted between the different species. According to the preliminary results on virulence factors, the risk of severe human infections by the detected livestock-associated MRSA strains seems to be low.

---

## 294-ZOP

### Analysis of the *Coxiella burnetii* isolate-specific pathogenic potential using proteomics

\*J. Andrack<sup>1</sup>, J. Dresler<sup>2</sup>, H. Neubauer<sup>1</sup>, K. Mertens-Scholz<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany

<sup>2</sup>Military Health Institute, Prague, Germany

Q fever is a disease caused by *Coxiella* (*C.*) *burnetii*, an obligate intracellular zoonotic pathogen, which affects humans and animals. In ruminants (e.g. sheep, goat and cattle) the infection is mainly asymptomatic, but can lead to abortions. In humans flu-like symptoms and pneumonia (acute) and/or endocarditis (chronic) may occur.

*C. burnetii* isolates are genetically heterogeneous and originally classified into six genomic groups. This classification correlates with an observed group-specific virulence, identified using rodent infection models. The pathogenic differences may be a result of a variable expression of virulence factors and predisposing factors of the individual host.

Our project is part of the Q-GAPS zoonosis network (**Q** fever **Ger**m**An** **I**nterdisciplinary **P**rogram for **re**Search) and focuses on isolate-specific differences in pathogenicity and virulence. The hypothesis of our work is that the genetic differences of various *C. burnetii* isolates correlates with differential expression of virulence factors. For determining the isolate-specific differences, the data from whole genome sequencing, cell culture infection models for replication fitness and corresponding proteome analysis will be compared. This will be used for the identification of protein markers for virulence determinants and may contribute to future development of new diagnostic tests and vaccines.

---

## 295-ZOP

Decolonization of LA-MRSA positive fattening pigs – Effects of straw bedding on conventional husbandry

\*S. Linnemann<sup>1</sup>, H. Müller<sup>1</sup>, I. Kobusch<sup>1</sup>, N. Effelsberg<sup>2</sup>, A.

Mellmann<sup>2</sup>, M. Boelhauve<sup>1</sup>

<sup>1</sup>South Westphalia University of Applied Sciences, Soest, Germany

<sup>2</sup>Institute of Hygiene, University of Münster, Münster, Germany

**Introduction:** The increasing occurrence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) requires a counterstrategy. MRSA decolonization of persons with livestock exposure was not successful, although both, environment of the animals and persons were decontaminated but colonized animals cause a reinfection.

**Objectives:** Through the #1HealthPREVENT project, factors could be worked out which cause a change of the MRSA-status of animals and environment.

**Materials and methods:** This research was performed on a conventional swine farm for fattening pigs. The feature of this conventional husbandry is the open airing and straw bedding. Two different types of hygienic concepts were compared: simple cleaning (SC) and cleaning with disinfection (CD). Nasal LA-MRSA colonization of the pigs was analyzed directly after arrival on the farm and subsequently during the fattening period. The environmental contamination of LA-MRSA was analyzed as well. Nasal swab of selected animals for microbiome analysis were taken.

**Results:** At arrival, all pigs carried LA-MRSA. Further screenings showed comparable results in the stables. After a few weeks, pigs and environment were frequently tested negative for LA-MRSA. Group SC showed a decrease of LA-MRSA-negative findings of pigs after 11 weeks with a total conversion to LA-MRSA negativity at the end of the fattening period after 14 weeks. This development also applies to Group CD. Microbiome analysis of five samples show that the microbiome changes during the fattening period.

**Conclusion:** The process of decontamination is not mainly influenced by the preparation of the stables. It seems like the straw bedding with a diverse microbiome could be an important factor. The microbiome analysis shows changes in the microbiome of the samples during the fattening period. Further analysis will likely give a hint for a better understanding of this interaction and might help developing an effective strategy against the spread of LA-MRSA.

---

## 296-ZOP

Identification of housing condition factors influencing the occurrence of MRSA and ESBL in pigsties

\*I. Kobusch<sup>1</sup>, S. Linnemann<sup>1</sup>, H. Müller<sup>1</sup>, M. Boelhauve<sup>1</sup>

<sup>1</sup>South Westphalia University of Applied Sciences, Germany, Soest, Germany

**Introduction:** Increasing evidence of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) and extended-spectrum lactamase-producing Enterobacteriaceae (ESBL) as multidrug-resistant microorganisms (MDRO) in animal husbandry is shifting research to the focus of public interest. It is known that animals can change their MRSA-status during their lifetime. However, other studies have shown a different prevalence of LA-MRSA in pig herds depending on environmental parameters.

**Objectives:** Aim of a subproject of #1HealthPREVENT is the identification of factors determining the spread of LA-MRSA and ESBL. The MDRO-prevalence in different pig husbandry

systems with divergent housing conditions is of special interest.

**Materials and methods:** The research was performed on fattening pig farms with conventional husbandry, alternative systems and husbandries with ecological guidelines. At two different times in the fattening period, nasal swabs (pigs) and sponge swabs (environment) were taken for the detection of MRSA and influencing factors were collected by a questionnaire. ESBL was screened in parallel by sock swabs.

**Results:** Conventional systems showed increased prevalence for LA-MRSA in environment and pigs. Alternative and ecological husbandries revealed reduced LA-MRSA findings when competitive flora like straw bedding or non-disinfection were present. ESBL is verified in all husbandry systems with a reduced rate in disinfected stables.

**Conclusion:** Results suggest a lower rate of LA-MRSA in alternative and ecological housing systems. Possibly, there could be created a complex environmental microbiota with bacterial competition against LA-MRSA. ESBL was verified in all systems, but with higher rates in intensive contact with faeces. The #1Health-PREVENT project investigates these influences for a better understanding of the interactions to define effective strategies to reduce MRDO occurrence in livestock breeding.

## 297-ZOP

Analysis of *Arcobacter butzleri* from water poultry: Insights into antibiotic-resistance and virulence determinants

\*E. Müller<sup>1</sup>, I. Hänel<sup>1</sup>, H. Hotzel<sup>1</sup>, M. Pfeffer<sup>2</sup>, H. Neubauer<sup>1</sup>, A. Busch<sup>3</sup>, H. Tomaso<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Bundesforschungsinstitut für Tiergesundheit, Institut für bakterielle Infektionen und Zoonosen (IBIZ), Jena, Germany

<sup>2</sup>Faculty of Veterinary Medicine, University of Leipzig, Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany

<sup>3</sup>University Hospital Jena, Department of Anesthesiology and Intensive Care Medicine, Jena, Germany

**Introduction:** *Arcobacter* is a genus of gram-negative, spiral-shaped bacteria in the class of epsilon proteobacteria. Some species are known as pathogens in both humans and animals. Therefore, they are of particular interest as zoonotic pathogens within the "One Health" framework.

**Objectives:** The purpose of this study was to analyze the distinct antimicrobial susceptibility of two *Arcobacter butzleri* strains isolated from ducks (*Cairina moschata* × *Anas platyrhynchos*) from a water poultry farm in Thuringia, Germany. We assessed their genetic relatedness, the antibiotic resistance-associated genes, and virulence-associated genes.

**Materials & Methods:** The isolates were cultivated, identified by MALDI-TOF MS and identification was confirmed with PCR. Antibiotic susceptibility testing was performed using the gradient strip method (E-Test™) with eight different antibiotics. Whole-genome sequences were generated with Illumina MiSeq instruments and examined with bioinformatic tools.

**Results:** Strain 16CS0817-2 was susceptible to all tested antibiotics except for streptomycin and cefotaxime. The other strain was only susceptible to gentamicin, ciprofloxacin, and ampicillin. A positive correlation between phenotype and genotype was found in three antimicrobial resistance-

associated genes for both strains. All virulence-associated genes were present in both isolates except for *hecA* and *hecB*.

**Conclusion:** Since the genotypic prediction of antibiotic resistance alone is not yet reliable, the phenotypic determination of antibiotic resistance should be maintained.

## 298-ZOP

Serological and molecular identification of *Brucella* spp. in pig from Cairo and Gizeh governorates, Egypt

\*A. U. Khan<sup>1</sup>, F. Melzer<sup>1</sup>, M. Elschner<sup>1</sup>, H. Neubauer<sup>1</sup>, H. El-Adawy<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany

**Background:** Brucellosis is considered as endemic disease of animals and humans since thousands of years in Egypt. However, brucellosis in pigs has never been reported. Thus, serological and molecular assays were applied to detect anti-*Brucella* antibodies and DNA in serum samples collected from pigs.

**Methods:** In total 331 blood samples collected from male and female pigs at slaughterhouses of Cairo and Giza governorates were investigated using *Brucella* c and i-ELISA and *Brucella* real time PCR.

**Results:** Anti *Brucella* antibodies were detected in 16 (4.83%) and 36 (10.8%) sera by i-ELISA and c-ELISA, respectively. *Brucella* DNA was detected in 10 (3.02%) seropositive samples and identified as *B. melitensis* (7/10) and *B. suis* (3/10). A higher prevalence was found in boars.

**Conclusion:** This is the first study investigating pig brucellosis in Egypt. The results of this study will arise awareness for brucellosis in these farm animals and will help to develop effective control strategies.

## 299-ZOP

A *Mycobacterium bovis* intrabronchial infection model established in goats

N. Wedlich<sup>1</sup>, J. Figl<sup>1</sup>, H. Köhler<sup>1</sup>, E. Liebler-Tenorio<sup>1</sup>, K. von Pückler<sup>2</sup>, S. Petow<sup>3</sup>, S. Mamerow<sup>1,4</sup>, M. Rissmann<sup>1</sup>, R. Ulrich<sup>4</sup>, P. Reinhold<sup>1</sup>, C. Schröder<sup>4</sup>, L. Grode<sup>5</sup>, S. H. E. Kaufmann<sup>6</sup>, \*C. Menge<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institute of Molecular Pathogenesis, Jena, Germany

<sup>2</sup>Justus-Liebig-University Giessen, Small Animal Clinic – Radiology, Giessen, Germany

<sup>3</sup>Friedrich-Loeffler-Institut, Institute of Animal Welfare and Animal Husbandry, Celle, Germany

<sup>4</sup>Friedrich-Loeffler-Institut, Department of Experimental Animal Facilities and Biorisk Management, Greifswald, Germany

<sup>5</sup>Vakzine Projekt Management GmbH, Hannover, Germany

<sup>6</sup>Max Planck Institute for Infection Biology, Berlin, Germany

*M. bovis* and *M. caprae*, members of the *Mycobacterium tuberculosis* complex (MTC), cause tuberculosis in domestic ruminants with large economic impact globally and also represent important zoonotic pathogens. Goats are well suited models for veterinary and human TB research as they develop symptoms and pathomorphological lesions comparable to bovine and human TB. Aim of the study was to implement a caprine TB infection model allowing for the quantitative assessment of a multitude of relevant parameters during the course of infection.

Three groups of 6 month old goats (n=4) were infected with  $4.7 \times 10^2$ ,  $8.9 \times 10^2$  and  $8.3 \times 10^3$  CFU of *M. bovis*,

respectively. Endoscopic inoculation was performed at 5 distinctive locations in the bronchial tree and progress of infection was monitored until 5 months post infection (p.i.). Humoral and cellular immune responses were assessed *ex vivo*. At necropsy, tissue samples were taken for bacterial culture, lungs fixed intra-thoracically and analyzed by Computed tomography (CT) and digital X-ray.

Although none of the goats developed clinical symptoms, macroscopic lung lesions were recorded in 1 of 4, 3/4 and 3/3 animals of the low, medium and high dose group, respectively. CT analysis unveiled lung lesions in numbers increasing with inoculation dose. Lesions presented as small mineralized lesions with < 5 mm in diameter, which predominated; mineralized lesions with >5 mm; confluent and partly mineralized lesions with >5 mm. *M. bovis* was regularly isolated from thoracic lymph nodes. Dissemination to lymph nodes at other sites, tonsils and the gastrointestinal tract accompanied with lesions was seen in four animals. Cross-reacting antibodies against other mycobacteria were detectable as early as 28 days p.i. (dpi) whereas antibodies specific for *M. bovis* antigens MPB70 and MPB83 became detectable only later and increases in titers were less pronounced. Antigen-specific IFN- $\gamma$  response of T-cells was measurable from 28 dpi onwards with CD4 $^{+}$ , CD8 $^{+}$  as well as  $\gamma\delta$ -T-cells contributing to IFN- $\gamma$  formation.

This large animal TB model by intra-bronchial inoculation can now be applied in anti-TB vaccine development.

### 300-ZOP

Multi-drug resistant gram-negative pathogens carried by flies from a tertiary hospital in Rwanda

S. Heiden<sup>1</sup>, M. Kurz<sup>2</sup>, J. Bohnert<sup>3</sup>, C. Bayingana<sup>4</sup>, J. Ndoli<sup>4,5</sup>, A. Sendegeya<sup>4,5</sup>, J. Gahutu<sup>4,5</sup>, \*E. Eger<sup>1</sup>, F. Mockenhaupt<sup>2</sup>, K. Schauler<sup>1</sup>

<sup>1</sup>University of Greifswald/Institute of Pharmacy, Pharmaceutical Microbiology, Greifswald, Germany

<sup>2</sup>Charité-Universitätsmedizin, Berlin, Germany

<sup>3</sup>University Medicine of Greifswald, Greifswald, Germany

<sup>4</sup>University of Rwanda, Kigali, Rwanda

<sup>5</sup>University Teaching Hospital of Butare, Butare, Rwanda

### Objektives

The rapid emergence of multidrug-resistant gram-negative (MRGN) bacteria is one of the major challenges of modern healthcare all around the globe. The carriage of such bacteria by ubiquitously occurring insects is an underrated risk factor.

### Material and Methods

In this study, 42 flies collected from different wards of a tertiary hospital in Rwanda were tested for their carriage of extended-spectrum beta-lactamase (ESBL)-producing bacteria and subsequently characterized by whole-genome sequencing.

### Results

Almost 48 % (n = 20) of samples contained phenotypically multidrug-resistant strains with ESBL-producing *E. coli* (n = 15, 36 %) as the dominating species. Furthermore, pathogenic *Acinetobacter baumannii*, *Enterobacter cloacae*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa* were found. Among the eight different *E. coli* sequence types (ST) were the pandemic clonal lineages ST131 and ST410. In addition to the frequently found resistance genes *bla*<sub>CTX-M-15</sub>, *bla*<sub>OXA-1</sub> and *aac*(3)-*Ila*, the biofilm formation-associated

virulence factor *csgD* such as genes encoding for adhesins (*cfaC*), haem-utilization (*chuA*) and toxins (*hlyE*) were identified.

### Conclusion

This study highlights the potential role of flies as reservoir for harmful antibiotic-resistant bacteria.

### 301-ZOP

Major *in vivo* marker of dermatophytoses demonstrated in *ex vivo* skin infection model

\*C. Baumbach<sup>1</sup>, J. Michler<sup>2</sup>, P. Nenoff<sup>3</sup>, S. Uhrlaß<sup>3</sup>, W. Schroedl<sup>1</sup>

<sup>1</sup>Leipzig University, Institute of Bacteriology and Mycology, Leipzig, Germany

<sup>2</sup>Leipzig University, Institute of Veterinary-Anatomy, Leipzig, Germany

<sup>3</sup>Laboratory of Medical Microbiology, Mölbis, Germany

### Introduction

Dermatophytoses, i.e. fungal infections of skin, nail and hair, are common worldwide and a growing health burden in animals and man. This high prevalence is connected to the dermatophytes' ability to degrade keratins through the secretion of exo- and endoproteases. However, an "ecological switching", i.e. the differential expression of various proteases depending on the environment, was noticed. The most abundant protease found in patient samples and animal experiments is Subtilisin 6 (Sub6).

### Objective

Demonstration of important *in vivo* disease markers in an *ex vivo* skin infection model and thereby proving this model system suitable for dermatophytoses research.

### Material and Methods

The employed *ex vivo* infection model is based on guinea pig skin explants (GPSE). Skin pieces were harvested from adult animals, disinfected and placed in 12-well cell culture inserts. Explants were supplemented with media; the experimental infection was induced by means of standardized conidia suspension of different *Trichophyton benhamiae*-isolates. Infected GPSE were incubated for 10 days (d), explant samples were taken on d3, 5, 7 and 10. The expression of the virulence factor Sub6 was assessed immunohistochemically using customized anti-peptide-antibodies and a modified catalyzed reporter deposition (CARD) detection system.

### Results

All applied *Trichophyton*-suspensions grew hyphae and infected the GPSE model successfully during the 10d culture. Dermatophytes proliferated well and a progressive destruction of the skin tissue was noticed. Sub6-expression was demonstrated immunohistochemically: the virulence factor is mainly found at the cell walls of fungal conidia and hyphae. Semiquantitative analysis revealed no statistical difference between isolates derived from human patients and infected GP.

### Conclusion

Here, we underlined the suitability of this skin infection model for dermatophytoses research by demonstrating the



expression of Sub6 during experimental *Trichophyton*-infection. The quantitative assessment of virulence factor expression is planned to enable the detailed comparison of different dermatophytes concerning these key mechanisms of infection.

---

### 302-ZOP

Metabolic traits of bovine STEC strains with different colonization properties

\*S. Barth<sup>1</sup>, M. Weber<sup>1</sup>, K. Schaufler<sup>2,3</sup>, C. Berens<sup>1</sup>, L. Geue<sup>1</sup>, C. Menge<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institut für molekulare Pathogenese, Jena, Germany

<sup>2</sup>University of Greifswald, Pharmaceutical Microbiology, Greifswald, Germany

<sup>3</sup>Free University Berlin, Institute of Microbiology and Epizootics, Berlin, Germany

Cattle can harbor Shiga toxin-producing *Escherichia coli* (STEC) in their intestinal tract without displaying any clinical symptoms but providing an ecological niche for these microorganisms. Genotypic characterization of bovine STEC isolates showed that their ability to colonize the animals' gut persistently (STECper) or only sporadically (STECspo) is more closely linked to the accessory rather than to the core genome (Barth et al., AEM, 2016, 82:5455). Phenotypic traits beyond classical virulence factors might be crucial for the realization of a specific colonization pattern. We therefore analyzed metabolic properties of 28 STEC strains, e.g. utilization of different single-compound carbon, nitrogen, phosphorous, and sulfur sources by phenotype microarray, in correlation to the colonization type. The ability to produce biofilm was determined quantitatively.

STECspo strains produced significantly more biofilm when incubated at lower temperatures than STECper. Three STECper strains belonging to the O165/O172 cluster utilized significantly less substrates when compared to the other strains and were excluded from further statistical analysis. The substrates showing significant association with the colonization type were, beside others, glyoxylic acid and L-rhamnose, which were utilized in STECspo, but not or only to a small extent in STECper. Comparing the genomic sequences of the respective *glc* and *rha* operons revealed mutations and frameshifts in relevant uptake and/or regulatory genes, particularly in STECper.

STECper seem to have abandoned metabolic traits after adaptation to the bovine intestine, while STECspo conserved properties leveraging survival in the environment. These findings, for the first time, link persistent colonization of STEC strains in the cattle reservoir to the loss of metabolic properties and the genomic mutations in the underlying genetic regulation. Not being classical virulence factors, these specific traits of strains with enhanced reservoir adaptation may represent a bacterial Achilles' heel to be targeted by novel intervention measures that aim to reduce long-term colonization of cattle with these potentially zoonotic pathogens.

---

### 303-ZOP

Tuberculosis in small animals living in close contact to their owners

\*S. Barth<sup>1</sup>, C. Menge<sup>1</sup>, F. Attig<sup>2</sup>, W. Baumgärtner<sup>2</sup>, A. Lehmbecker<sup>2</sup>, M. Kohlbach<sup>3</sup>, D. Hillemann<sup>4</sup>, A. Lauda<sup>5</sup>, S. Pflieger<sup>6</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institut für molekulare Pathogenese, Jena, Germany

<sup>2</sup>Tierärztliche Hochschule Hannover, Department of Pathology, Hannover, Germany

<sup>3</sup>Veterinary Joint Practice Janssen and König, Wennigsen, Germany

<sup>4</sup>National Reference Centre for Mycobacteria, Borstel, Germany

<sup>5</sup>Veterinary Practice Dres. Schwarz and Lauda, Planegg, Germany

<sup>6</sup>Practice for Animal Pathology, München, Germany

*Mycobacterium bovis* is the causative agent of bovine tuberculosis, but also has zoonotic potential causing diseases in man and several other mammal species. Here we describe two independent cases of *M. bovis* infections, one in a cat and one in a ferret, both small animals living in the house-hold in close contact to the owners.

Case 1 was a female, 8-month-old domestic short hair cat with wound complications after castration surgery in Ukraine. A second surgery in Germany showed a focal, partly cystic mass within the mesentery. Macroscopically, several abdominal lymph nodes were enlarged. Case 2 was an intact, 9-month old female pet ferret, presented to a local veterinarian with symptoms of weight loss, apathy, and hyporexia. Explorative laparotomy identified a firm mass of approximately 2x2 cm in size in the mesentery of the jejunum. The ferret was bought in Poland and lived eight months in Germany before the infection became apparent.

Histological examinations of the abdominal masses of both animals detected acid fast bacteria laying in the cytoplasm of macrophages, leading to the suspicion of an infection with *Mycobacteria* sp.. PCR confirmed presence of DNA of members of the *Mycobacterium tuberculosis* complex, subsequently specified as *M. bovis*. Molecular genetic typing identified *M. bovis* spoligotypes never reported before in Germany (SB0950, SB2548).

The current cases must be taken as warning signals to raise the awareness of veterinarians and physicians for mycobacterial infections in small animals, as animal tuberculosis is rare in wide areas of Germany and generally not considered a differential diagnosis.

---

### 304-ZOP

Antimicrobial-resistant pathogens transmitted via pets

\*C. Hackmann<sup>1</sup>, P. Gastmeier<sup>1</sup>, R. Leistner<sup>1</sup>

<sup>1</sup>Charité Universitätsmedizin Berlin, Institut für Hygiene und Umweltmedizin, Berlin, Germany

This project aims to assess the relevance of pet husbandry in the transmission of multidrug-resistant (MDR) pathogens on hospital patients. Currently, the potential role of pets as reservoirs of MDR pathogens is still unclear. The project focusses on the most common MDR pathogens in pet owners, namely methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE) and multidrug-resistant gram-negative pathogens (MRGN). Investigating pet husbandry as a risk factor for colonization or infection with MDR pathogens creates an opportunity to identify patients at risk and develop prevention strategies.

To assess the contact to household pets as a risk factor for the colonization or infection with one of the above named pathogens, we perform an exploratory, unmatched case-control-study. Among questions about well-known risk factors, study participants are interviewed regarding their contact especially to dogs and cats. This includes information about the number of pets in the household, the closeness of contact and diseases as well as medical treatment of the pets.

To assess the genetic relatedness of the MDR pathogens between humans and pets we collect nasal and rectal swabs of the participants in the hospital to test them for MDR

pathogens. We ask owners of dogs or cats to collect nasal and rectal swabs of their pets as well and send them back to us for further analysis. Phenotypically matching MDR pathogens in the samples of participants and their pets will be tested for genetic relatedness using whole genome sequencing.

The study is performed at the Charité - University Hospital Berlin. The data collection phase started in June 2019 and will continue until May 2021. The sample size will comprise 4.000-6.000 participants.

---

### 305-ZOP

Interaction of *Escherichia coli* Shiga toxins with glycosphingolipid receptors of porcine renal epithelial cells  
\*J. Detzner<sup>1</sup>, C. Gloerfeld<sup>1</sup>, N. Legros<sup>1</sup>, G. Pohlentz<sup>1</sup>, H. U. Humpf<sup>2</sup>, A. Mellmann<sup>3</sup>, H. Karch<sup>3</sup>, J. Muthing<sup>1</sup>

<sup>1</sup>University of Muenster, Institute for Hygiene, Glycobiology Group, Münster, Germany

<sup>2</sup>University of Muenster, Institute of Food Chemistry, Münster, Germany

<sup>3</sup>University of Muenster, Institute for Hygiene, Münster, Germany

**Introduction:** Certain pathogenic strains of Shiga toxin (Stx) producing *Escherichia coli* (STEC) cause the porcine edema disease that occurs shortly after weaning. The swine-pathogenic STEC strains release the Stx2e subtype as their key virulence factor. Stx2e damages endothelial cells of a number of animal organs including the kidney. The interaction is mediated by the Stx-binding glycosphingolipids (GSLs) globotriaosylceramide (Gb3Cer) and globotetraosylceramide (Gb4Cer). So far, the involvement of renal epithelial cells in Stx2e-mediated injury of the swine kidney is unknown.

**Objective:** The major aims were the identification of Stx2e-binding GSLs and determining the Stx2e-mediated damage of porcine kidney epithelial cells.

**Materials and Methods:** The porcine PK15 and LLC-PK1 kidney epithelial cell lines were cultured under serum-free conditions. The neutral GSLs were isolated from lipid extracts by anion-exchange chromatography. Antibody and Stx thin-layer chromatography (TLC) overlay assays were applied for immunochemical detection of Stx-binding GSLs. Their precise structures were identified by mass spectrometry. Neoglycolipids, composed of Gb3 and Gb4 glycans linked to phosphatidylethanolamine (PE), respectively, were produced and probed as potential Stx2e inhibitors in cytotoxicity assays.

**Results:** PK15 and LLC-PK1 cells were found to exhibit the two Stx receptors Gb3Cer and Gb4Cer. Gb4Cer, the preferential receptor of Stx2e, dominated over Gb3Cer in both cell lines. TLC overlay immunochemical detection revealed strong binding of Stx2e towards Gb4Cer compared to weak recognition of less abundant Gb3Cer. This binding preference was different to that of human-pathogenic Stx1a and Stx2a, which preferred Gb3Cer. Co-application of the neoglycolipids Gb3-PE and Gb4-PE in cytotoxicity assay increased the survival rate upon Stx2e treatment of both cell lines indicating the potential of neoglycolipids as Stx inhibitors.

**Conclusion:** In this study we provide first data on Gb3Cer and Gb4Cer as main GSLs of porcine epithelial cells and Stx2e-mediated cellular damage. Our results suggest involvement of the kidney epithelium in the edema disease of pigs.

---

### 306-ZOP

Tick feces as a potential infection source of Q fever examined in an *in vitro* feeding system

\*S. Körner<sup>1,2</sup>, G. R. Makert<sup>2</sup>, K. Mertens-Scholz<sup>1</sup>, K. Henning<sup>1</sup>, M. Pfeffer<sup>3</sup>, S. Ulbert<sup>2</sup>

<sup>1</sup>Friedrich-Loeffler-Institute for Bacterial Infections and Zoonoses, Jena, Germany

<sup>2</sup>Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, Germany

<sup>3</sup>Institute of Animal Hygiene and Veterinary Public Health, Leipzig, Germany

*Coxiella burnetii* is an intracellular replicating bacterium that may cause the zoonosis Q fever in a wide range of hosts. Domestic ruminants are the most relevant reservoir for human infection whereby transmission mainly occurs via inhalation of infected dusts. Since the first isolation from a tick, Q fever was also assumed to be tick-borne. However, ticks carrying this pathogen are found rarely and close related *Coxiella*-like endosymbionts could have caused an overvaluation. Thus, the significance of ticks in Q fever transmission is unclear.

To address open questions concerning the vector role of ticks, different routes of tick excretion were examined in an *in vitro* feeding system.

Adult *Ixodes ricinus* and *Dermacentor marginatus* were fed on blood inoculated with different concentrations of *C. burnetii* in silicone-membrane based feeding units. Tick feces was removed daily and tested by quantitative real-time PCR and cultivation in L-929 cells. In order to investigate transstadial transmission, *I. ricinus* nymphs were fed on inoculated blood and left for molting. One part of molted adults was tested by qPCR whereas the other part was fed again on sterile blood for testing excretion via saliva as well via feces.

When feeding on blood inoculated with 10<sup>5</sup> or 10<sup>6</sup> *C. burnetii* GE/ml, adult ticks excreted infectious *C. burnetii* in feces especially one week after inoculation. Transstadial transmission from *I. ricinus* nymphs to adults occurred in 25 % of ticks. Some of the molted females, which were fed again on sterile blood, excreted feces containing *C. burnetii* DNA during the entire duration of feeding in amounts of up to 10<sup>6</sup> *C. burnetii* GE/mg feces. The blood, which was removed during blood changes, was tested negative.

*D. marginatus* and *I. ricinus* adults can excrete infectious *C. burnetii* when feeding on blood containing a sufficient concentration of bacteria. This demonstrates an infection risk by inhalation of contaminated tick feces. Transstadial transmission was observed under laboratory conditions, proving a potential vector competence of *I. ricinus*. The role of the tick bite as a further potential transmission pathway still needs to be investigated.

---

### 307-ZOP

**Project ZooM - Zoonotic importance of multidrug-resistant pathogens: FAQs at the interface veterinary/human medicine**

\*S. Kutzora<sup>1</sup>

<sup>1</sup>Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, München, Germany

In recent years, the number of infections caused by multidrug-resistant bacteria (MDRB) has increased. MDRB are also increasingly important in the private sector (e.g.

companion animals), but this aspect has not been sufficiently addressed in previous recommendations. In addition, many persons (e.g. farmers, veterinarians and their household members) acquire MDRB through contact with farm animals. The guidelines of the Commission for Hospital Hygiene and Infection Prevention (KRINKO) at the Robert Koch-Institute as well as the Technical Rules for Biological Agents (TRBA) in Germany focus on recommendations for healthcare facilities for the prevention of nosocomial or occupational infection risks. There are no recommendations for the private sector or for non-medical facilities and institutions. Consequently, in the public health service, answers regarding many questions related to (zoonotic) MDRB in these sectors are lacking. The first aim of the ZooM project is to identify open questions relevant to the public health service regarding the zoonotic meaning of MDRB at the interface between veterinary and human medicine. Afterwards, information on possible transmission pathways of MDRB between humans and animals will be presented as FAQs. In order to generate the FAQs, guideline-based expert interviews were conducted. For this purpose, interviews were carried out on five different occasions. From the resulting pool of questions, overarching central questions were formed and answered with the help of scientific literature and an interdisciplinary panel of experts. Subsequently, the FAQs and their dissemination will be evaluated with regard to their benefit/usefulness for the daily work of the public health service (human and veterinary medicine). The ZooM project is funded by the Federal Ministry of Education and Research (BMBF) as part of the Zoonotic Infectious Diseases Research Network.

---

### 308-ZOP

Investigations into coinfections of the obligate intracellular ruminant pathogens ***Chlamydia abortus*** and ***Coxiella burnetii***

B. Sara<sup>1</sup>, E. Liebler-Tenorio<sup>1</sup>, \*C. Schnee<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut, Institut für molekulare Pathogenese, Jena, Germany

*Chlamydia abortus* and *Coxiella burnetii* are

obligate intracellular gram negative bacteria that infect small ruminants.

Both target the placenta, can cause abortion and possess a zoonotic

potential. *Chlamydia* and *Coxiella* share even more striking similarities on

the cellular and molecular level such as a biphasic life-cycle with

extracellular, infectious variants and intracellular, non-infectious forms

residing in a membrane-bound vacuole. By hijacking intracellular organelles

and redirecting transport vesicles, the bacteria acquire essential nutrients,

but in a different mode. While the vacuole of *Coxiella* is an acidified

phagolysosome which fuses with endocytic vesicles, *Chlamydia* in non-acidified

inclusions receives its nutrients from fusiogenic events with exocytic

vesicles from the Golgi apparatus and the endoplasmic reticulum. Field

studies in small ruminants have shown coinfections of *Chlamydia* and *Coxiella*

in placental tissue from abortions. We have screened 65 placenta samples

collected after normal parturition from infected sheep flocks. 52.3 % of

these samples were PCR-positive for *Chl. abortus*, 61.5 % for *Cox. burnetii*

and in 40.0 %, a coinfection of both agents was detected. To investigate

whether the interaction of the two pathogens is of synergistic, competitive

or neutral nature and to better assess the contribution of such polymicrobial

infections to disease progression, we analyzed the interaction of *Chl.*

*abortus* DC59 and *Cox. burnetii* RSA 439 NMII in cell culture models.

Fluorescence and electron microscopy revealed that different cell lines can

be coinfecting with *Coxiella* and *Chlamydia* and that a single cell can harbour

both pathogens. They reside in distinct vacuoles but in close proximity to

each other with occasional fusion of vacuole membranes. A reinfection of

cells with *Coxiella* does not alter general *Chlamydia* morphology, but growth

and infectivity was negatively influenced as shown by qPCR analysis of DNA

replication and titration.

---

### 309-ZOP

The author has not agreed to a publication.

---

### 310-ZOP

Review on selected bacterial zoonoses in Bulgarian citizens: 2004-2018

R. Nenova<sup>1</sup>, I. Tomova<sup>1</sup>, I. Ivanov<sup>1</sup>, \*S. Panaiotov<sup>1</sup>, V. Tolchkov<sup>1</sup>

<sup>1</sup>National Center of Infectious and Parasitic Diseases, Department of Microbiology, Sofia, Bulgaria

**Objective:** An overview on anthrax, tularemia and brucellosis cases in Bulgarian citizens for 15 years.

**Materials and methods:** Data from the National Reference Laboratory "Special bacterial Pathogens", Microbiology department, NCIPD, Bulgaria.

**Results:** Nineteen cases of anthrax (17 sporadic and 2 clustered), including 3 fatal, were reported. With the exception of one primary septic form, the rest of the patients were with typical cutaneous anthrax. For the above mentioned period 91 patients with tularemia were diagnosed. These data indicate a change in the nature of the epidemic process since its beginning in 1997: reducing of the intensity and spreading of the infection to new regions in the country. Oro-pharyngeal, glandular and rarely oculo-glandular and pulmonary clinical presentations were observed. During the last 15 years 178 humans were infected with brucellosis in Bulgaria, including imported cases, 2 autochthonous outbreaks and autochthonous sporadic cases. Classical microbiological methods, such as cultivation and direct immunofluorescence microscopy were used for the diagnosis of anthrax. For the other infections, specific antibody response was the main method for diagnosis. Isolation of *B. melitensis* was successful from 10 clinical samples and *F. tularensis* from 4 samples. Molecular methods (real-time qPCR, MLVA, Bruceladder) were used for diagnostic purposes, as well as for typing of the isolates.

**Conclusion:** Human anthrax cases still occurred in enzootic Bulgarian regions. Autochthonous cases of tularemia and brucellosis re-emerged in Bulgaria. The effective infection control led to incidence reduction in the past several years. However, the possibility *B. anthracis*, *F. tularensis* and *Brucella* sp. to be used as bioterroristic agents requires our vigilance during the investigation of human infections caused by them.

**Key words:** human zoonoses, anthrax, tularemia, brucellosis

### 311-ZOP

One year follow-up of patients with acute Q fever: results from an outbreak in Southwest Germany, 2014-2015  
L. Dangel<sup>1</sup>, \*S. Fischer<sup>1</sup>

<sup>1</sup>Landesgesundheitsamt Baden-Württemberg, Stuttgart, Germany

### Background:

In July 2014, a Q fever (QF) outbreak with over 250 notified cases occurred among visitors of a farmers' market with sheepfold in rural Southwest Germany. The outbreak gave the special opportunity to screen a large number of patients over a long period, who have been infected with one natural point source.

### Methods:

In this cohort, IgG PhI/II antibodies against *Coxiella burnetii* were analysed using IFA at defined time points as follow-ups. Twelve months post infection (p.i.) a short fatigue-based-questionnaire was conducted.

### Results:

Highest IgG PhI/II antibody titers were detected 3 months p.i. and persisted at this level until 9 months p.i.. Still, 12 months p.i. 76.2% of patients revealed high IgG PhII antibody titers. IgG PhI antibody titers were also increased 12 months p.i. in

18% of patients. One patient was identified with chronic QF on the basis of serological and clinical testing. Furthermore a high number (40%) of patients complained of fatigue symptoms 12 months p.i..

### Conclusions:

Our study clearly demonstrates the importance of long-term antibody surveillance after acute Q fever. At least 3, 6 and 12 months p.i. patients should be monitored for chronic QF. Therefore our study shows that both serology and clinical symptoms should be analysed for the detection of long-term health impacts such as chronic QF and fatigue symptoms. Due to these possible long-term consequences, prevention of *Coxiella*-infection, such as animal vaccination, is highly recommended.

### 312-MSHP

#### Antitumor anthraquinones from an Easter Island sea anemone: animal or bacterial origin?

I. Sottorff<sup>1</sup>, \*J. Wiese<sup>1</sup>, S. Künzel<sup>2</sup>, M. Lipfert<sup>3</sup>, N. Preußke<sup>3</sup>, F. D. Sönnichsen<sup>3</sup>, J. F. Imhoff<sup>1</sup>, U. Hentschel<sup>1</sup>

<sup>1</sup>GEOMAR Helmholtz Centre for Ocean Research Kiel, Marine Symbioses, Kiel, Germany

<sup>2</sup>Max Planck Institute for Evolutionary Biology, Plön, Germany

<sup>3</sup>Otto Diels Institute for Organic Chemistry, Kiel, Germany

The presence of two known anthraquinones, Lupinacidin A and Galvaquinone B, which have antitumor activity, has been identified in the sea anemone (*Gyactis sesere*) sampled from the coastal zone of Easter Island [1]. So far, these anthraquinones have been characterized from terrestrial and marine Actinobacteria only. In order to identify the anthraquinones producer, we isolated Actinobacteria associated with the sea anemone and obtained 28 representatives of seven actinobacterial genera, i.e. *Arthrobacter* (11 strains), *Cellulosimicrobium* (2), *Dietzia* (2), *Micromonospora* (3), *Rhodococcus* (1), *Streptomyces* (2), and *Verrucosipora* (7) [2]. Studies of cultures of these bacteria by HPLC, NMR, and HRLCMS analyses showed that the producer of lupinacidin A and galvaquinone B indeed was one of the isolated Actinobacteria [1]. The producer strain, SN26\_14.1, was identified as a representative of the genus *Verrucosipora*. Genome analysis of this strain revealed, that the production of the identified metabolites apparently follows a recently characterized PKS type II pathway with a Baeyer-Villiger type rearrangement assembly line.

Our study adds *Verrucosipora* as a new genus to the anthraquinone producers, in addition to well-known species of *Streptomyces* and *Micromonospora*. By a cultivation-based approach, the responsibility of symbionts of a marine invertebrate for the production of complex natural products found within the animal's extracts could be demonstrated. This finding re-opens the debate about the producers of secondary metabolites in sea animals. Finally, it provides valuable information about the chemistry of bacteria harbored in the geographically-isolated and almost unstudied, Easter Island.

### References

[1] Sottorff I, Künzel S, Wiese J, Lipfert M, Preußke N, Sönnichsen FD, Imhoff JF. Antitumor anthraquinones from an Easter Island sea anemone: animal or bacterial origin? Marine Drugs 2019;17:154.

[2] Sottorff I, Wiese J, Imhoff JF. High diversity and novelty of Actinobacteria isolated from the coastal zone of the

### 313-MSHP

#### Elucidating the gut microbiome–metabolome correlations in Wistar rats

\*A. Murali<sup>1</sup>, H. J. Cameron<sup>2</sup>, V. Giri<sup>1</sup>, N. R. Habermehl<sup>1</sup>, C. Behr<sup>3</sup>, S. Sperber<sup>1</sup>, T. Walk<sup>4</sup>, B. van Ravenzwaay<sup>1</sup>

<sup>1</sup>BASF SE, Experimental Toxicology, Ludwigshafen am Rhein, Germany

<sup>2</sup>BASF Plant Science LP, North Carolina, United States

<sup>3</sup>University College Cork, Biosciences Institute, Cork, Ireland

<sup>4</sup>BASF Metabolome Solutions GmbH, Berlin, Germany

The gut microbiome is known to play a huge role in the health and development of the host organism. "Omics technologies" provide better understanding of gut microbial functionality. The correlation between gut microbial communities with changes in the metabolome of plasma, feces and caecum matrices following a 28-day oral study with 6 antibiotics (streptomycin, vancomycin, roxithromycin, sparfloracin, lincomycin and clindamycin) in rats was investigated.

Analyses of the 16S data of the antibiotic treatments were performed using QIIME 2. A predictive functional profiling was developed to estimate the metagenomic composition of the 16S dataset using PICRUSt software. The correlation of respective results regarding the presence and absence of specific bacterial communities and/or genes with the metabolite levels in different treatments using the programming language R.

Antibiotics were observed to specifically alter the levels of lipids and fatty acid, bile acid and amino acid metabolism. Specifically, Hippuric acid and indole-3-acetic acid were strongly downregulated. Drug-specific influences were observed on the gut flora composition and the two lincosamides show very similar response, confirming a class-dependent effect. Streptomycin treatment showed similar bacterial abundance as the controls, dominated by Bacteroidetes and Firmicutes families. Vancomycin and Sparfloracin showed reduced Bacteroidetes and Proteobacteria families while the two lincosamides showed increased Firmicutes and Proteobacteria levels. Lincomycin showed dose-dependent alterations in Actinobacteria family levels. PCoAs of microbiome data showed clustering similar to the PCA of the metabolome data, indicating dependence of various metabolite levels on the gut community. Stronger correlations between the gut flora composition with the caecum and feces metabolome profiles of the antibiotic treated rats have been observed, compared to plasma. Differential gene regulation with respect to antibiotic treatments was observed and could be associated with the metabolite profiles. Hence, the gut microbial composition but also gene regulation profile could be correlated with the metabolome data.

### 314-MSHP

#### Characterization of oral bacteriome dysbiosis in type 2 diabetes

\*M. K. Azim<sup>1</sup>, F. Saleem<sup>1</sup>, J. Kori<sup>1</sup>

<sup>1</sup>Mohammad Ali Jinnah University, Biosciences, Karachi, Pakistan

#### Question

We aimed to characterize the relationship of oral microbiome with diabetes in Pakistan.

#### Methods

Saliva samples were collected from diabetic patients (n = 49) and healthy individuals (n = 55). 16S metagenomics saliva was carried out by next generation DNA sequencing.

#### Results

We observed that the phylum *Firmicutes* (p-value = 0.024 at 95% confidence interval) was significantly more abundant among diabetic patients than among the controls. We found that abundance of phylum *Actinobacteria* did not significantly vary among both groups in contrast to a similar report from USA (Long et al., 2017). On genus level, acidogenic bacteria *Prevotella* (p-value = 0.024), *Veillonella* (p-value = 0.013) and *Laptotrichia* (p-value =  $1.5 \times 10^{-3}$ ) were found to be in higher abundance in diabetic patients. Stratified analysis by gender revealed healthy and diabetic females to be more divergent. Abundance of *Prevotella* (p-value =  $4.4 \times 10^{-3}$ ) and *Laptotrichia* (p-value = 0.015) was significantly associated with male patients.

#### Conclusion

Comparison of oral bacteriome between two groups revealed dominance of acidogenic bacteria in diabetics. These bacteria are found in dental biofilm and produce organic acid(s) from fermentable carbohydrates. Significant abundance of acidogenic bacteria suggested involvement of these eubacteria in periodontal diseases in diabetic patients.

### 315-MSHP

#### Towards an Antibiotic-free Antimicrobial Therapy of Skin Disorders by Applying Probiotics

G. Khalfallah<sup>1</sup>, E. Heine<sup>1</sup>, \*R. Lütticken<sup>1</sup>

<sup>1</sup>DWI Leibniz-Institute for Interactive Materials, Aachen, Germany

**Introduction:** Many skin disorders, including acne and infected neurodermitis lesions, require topic and/or systemic antibiotic treatment. This also applies for other skin lesions like infected wounds or venous leg ulcers.

**Objectives:** The potential of certain lactic acid bacteria (probiotics) was evaluated with respect to their activity against bacterial skin pathogens. The aim of the study was to develop a plaster/bandage utilizing inhibitory substances produced by probiotics.

**Materials & Methods:** Two *S. salivarius* strains and one *L. plantarum* were employed as model probiotics. Their ability to produce antimicrobials (bacteriocin-like substances) active against skin and wound pathogens was tested using established methods. A newly designed membrane test was applied to secure that the probiotics produce antimicrobials diffusible through semipermeable membranes. Target organisms investigated were (among others) *C. acnes*, *S. aureus*, *P. aeruginosa*, and *Enterobacteriales*. For the purpose of a "probiotic pad" (bandage) to be applied onto the skin, the probiotics were cultivated in suitable media and transferred with the addition of a protectant into a pouch made of two sealed polymer films, one tight and one semipermeable. After vacuum-drying, the probiotics enclosed in the pads were tested on agar surfaces for their antimicrobial activity against the skin pathogens.

**Results:** The probiotics used were active against all skin pathogens tested. Moreover, these probiotics could be enclosed between polymer membranes – one tight for

bacteria, the other permeable for their products, preserved by vacuum drying, and reactivated after prolonged storage by applying them onto agar – as a model for their potential application onto skin. The reactivated polymer-pads demonstrated antibacterial activity on agar against all pathogens tested – including antibiotic-resistant isolates of *S. aureus*, *K. pneumoniae*, and *P. aeruginosa*.

**Conclusion:** Our results suggest that the probiotics containing pads might be topically applied for the treatment of skin disorders, e. g. acne, without the need for a regular antibiotic treatment or as an adjunct supporting antibiotic therapy.

---

### 316-MSHP

#### Towards the role of primary metabolites in the intestinal ecosystem under normal and inflamed conditions

\*A. Weiß<sup>1</sup>, D. Ring<sup>1</sup>, W. Eisenreich<sup>2</sup>, K. Jung<sup>3</sup>, B. Stecher<sup>1</sup>

<sup>1</sup>Ludwig-Maximilians-Universität, Max von Pettenkofer-Institut, Munich, Germany

<sup>2</sup>Technische Universität München, Biochemistry, Munich, Germany

<sup>3</sup>Ludwig-Maximilians-Universität, Microbiology, Munich, Germany

The composition of the mammalian gastrointestinal microbiome is not only determined by stochastic processes such as dispersal or ecological drift, but is strongly influenced by deterministic interactions between species and the individual host environment. In the healthy gut, the enteric microbiota faces frequent changes in the availability of nutrients, as the source of metabolites varies in space and time with dietary composition, as well as with host-dependent factors. Especially inflammatory processes can create metabolic niches in the gut that may affect the composition of available nutrients. Therefore, complex metabolic interaction networks between the bacterial species have evolved in the dynamic chemical environment of the mammalian gut, shaping the composition and function of the microbial ecosystem.

A prime example of bacterial metabolic interaction is syntrophic cross-feeding of primary gut luminal metabolites, specifically short chain fatty acids and intermediate reaction products as lactate, hydrogen and pyruvate. To gain a better understanding of the spatial, temporal and compositional complexity of metabolic microbial community interactions, we are investigating the role of primary metabolites in a defined subset of community members *in vitro*, as well as in mouse models of gut inflammation, using growth and metabolome profiling approaches. To this end, we are working with a synthetic bacterial consortium, the Oligo-MM12 consortium, a defined and well-characterized community of 12 commensals. Using experimental and theoretical approaches, we aim to identify primary metabolites being exchanged and keystone species dominating community interactions under normal conditions and during infection with pathogenic organisms, as *Salmonella enterica* serovar Typhimurium.

---

### 317-MSHP

#### Elucidation of the impact of (co)-infections on gut microbiome structure and function by metaproteomics

\*L. C. Gierse<sup>1</sup>, A. Meene<sup>1</sup>, C. Wünsche<sup>1</sup>, D. Zühlke<sup>1</sup>, N. Siemens<sup>2</sup>, D. Becher<sup>1</sup>, T. Urich<sup>1</sup>, K. Riedel<sup>1</sup>

<sup>1</sup>Institute of Microbiology, University of Greifswald, Greifswald, Germany

<sup>2</sup>Institute for Genetics and Functional Genomics, University of Greifswald, Greifswald, Germany

Pathogenicity models like swine and mouse play an important role in modern medical research. The large similarity to humans in physiology, immune system and biomass, makes especially the swine an ideal model for infectious diseases, e.g. influenza A or Staphylococcal infections. In contrast to most studies only addressing microbiome composition by using 16S rRNA gene sequencing, we aim to elucidate the structure and function of the metabolically active gastrointestinal tract microbiome prior and after bacto-viral mono- and co-infections employing a metaproteomics approach.

As a starting point a robust metaproteomic pipeline has been established, which enabled us to identify more than 5000 protein groups for each fecal sample of mouse and swine.

In healthy swine most of these protein groups belong to the families *Prevotellaceae* (35%), *Lactobacillaceae* (12%), *Lachnospiraceae* (12%) and *Clostridiaceae* (10%). While longitudinal shifts in the healthy gastrointestinal microbiota on the taxonomical level (e.g. *Spirochaetaceae* and *Streptococcaceae*) were observed, most functions stayed stable over a time span of 30 days, indicating functional redundancy. Notably, more than 60% of the protein groups could be assigned to major functional categories, such as translation (28%), carbohydrate transport and metabolism (6%), energy production and conservation (4%), or cell motility (4%). After influenza A infection of swine changes within the relative abundance of several families of the porcine gut microbiota e.g. *Lactobacillaceae*, *Veillonellaceae* and *Streptococcaceae* were observed, suggesting a systemic effect of the respiratory tract infection. Next, structure and function of the gastrointestinal microbiome will be analysed in response to Streptococcal and bacto-viral co-infections by our metaproteomics approach.

In the mouse model, nasal colonization with *S. pneumoniae* does not significantly affect the metaproteome of the intestinal microbiome. We are currently analyzing the effect of respiratory *S. pneumoniae* infections on the mouse gastrointestinal tract microbiome. First results indicate a reduction of microbial diversity in diseased animals compared to healthy controls.

---

### 318-MSHP

#### Establishing an *in-vitro* co-cultivation system to analyze interaction of biofilms and human endothelial cells

\*V. I. Pauker<sup>1</sup>, L. Rieckmann<sup>1</sup>, T. Petscheleit<sup>1</sup>, L. Maletzki<sup>2</sup>, A.

Strohbach<sup>2</sup>, R. Busch<sup>2</sup>, S. Illner<sup>3</sup>, N. Grabow<sup>3</sup>, K. Riedel<sup>1</sup>

<sup>1</sup>Institute of Microbiology, University of Greifswald, Greifswald, Germany

<sup>2</sup>University Medicine Greifswald, Clinic und Policlinic for Internal Medicine B, Researchcluster III, Greifswald, Germany

<sup>3</sup>Institute for Biomedical Engineering, University Medicine Rostock, Rostock, Germany

**Question-** Biofilm formation on cardiovascular implants can lead to life-threatening conditions such as infective endocarditis, short IE. The major cause of IE are bacteria of the genera *Streptococcus*, *Staphylococcus* and *Enterococcus*, which colonize the implants and are able to form biofilms. After implantation of the device a race between reendothelialization and biofilm formation begins. Infections of cardiovascular implants can lead to life threatening conditions and early diagnosis of these infections is a prerequisite for an effective treatment. Thus, this study aimed to establish an *in-vitro* co-cultivation system for biofilms and human endothelial cells in order to determine biofilm-relevant and species-specific proteins that can be used as diagnostic marker.

**Methods-** A micro-titer assay with cultivation inserts was established for an indirect co-cultivation of human coronary artery endothelial cells (HCAEC) with different strains of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. An LC-MS<sup>E</sup>-based proteomics approach was used to record first protein signatures of *S. aureus* biofilms in co-cultivation with human endothelial cells. A flow-through system with subsequent confocal laser-scanning microscopy was used for a qualitative evaluation of the biofilm formation on human endothelial cells.

**Results and conclusion-** The use of cultivation inserts prolonged the survival time of HCAEC in co-cultivation with the bacteria and assured formation of mature biofilms. First proteomic analysis revealed differential expression of proteins associated with virulence and protein metabolism. The flow-through system will be further used in order to evaluate possible compounds as possible coating substances for endothelialized TAVI surfaces.

---

### 319-MSHP

Impact of salinity on the gastrointestinal bacterial community of the snail *Theodoxus fluviatilis*

C. Kivistik<sup>1</sup>, J. Knobloch<sup>2</sup>, K. Käiro<sup>1</sup>, V. Kisand<sup>1,3</sup>, H. Tammert<sup>1</sup>, J. P. Hildebrandt<sup>2</sup>, \*D. Herlemann<sup>1</sup>

<sup>1</sup>Estonian University of Life Sciences, Centre for Limnology, Vehendi küla, Estonia

<sup>2</sup>University of Greifswald, Zoological Institute and Museum, Greifswald, Germany

<sup>3</sup>University of Tartu, Institute of Technology, Tartu, Estonia

Differences in salinity are boundaries acting as barriers for dispersal for many organisms, including bacteria. This creates distinctive biota on freshwater vs brackish water (mesohaline) environments. To test how saline boundaries influence the diversity and composition of host-associated microbiota we analyzed the digestive tract microbiome of *Theodoxus fluviatilis*, an organism able to cross the freshwater and mesohaline boundary. Alpha-diversity measures in the samples were indifferent between freshwater vs brackish water. However, the bacterial community composition of the freshwater *T. fluviatilis* microbiome were significantly different compared with mesohaline *T. fluviatilis* and typical bacteria could be determined for the freshwater and the mesohaline digestive tract microbiomes. An artificial increase of salinity for freshwater snails resulted in a strong change of the bacterial community and typical marine bacteria became part of the digestive tract microbiome of the freshwater *T. fluviatilis*. In contrast to freshwater snails, the mesohaline snails digestive tract microbiome composition did not change cardinally after the salinity increase/decrease. In all samples *Pseudomonas*, *Pirellula*, *Flavobacterium*, *Limnohabitans* and *Acinetobacter* were among the most abundant bacteria. These bacterial genera were largely unaffected by environmental conditions and as permanent members of the gut microbiota in *T. fluviatilis* they may support the digestion of the algal food. Our results show that freshwater and mesohaline water host-associated microbiomes respond differently to changes in salinity. Therefore salinization of coastal freshwater environments due to sea level rise can influence the gut microbiome and its functions with currently unknown consequences for the host.

---

### 320-MSHP

Analysis of skin surface decontamination methods to assess unbiased tick-borne microbiomes

\*A. Hoffmann<sup>1</sup>, V. Fingerle<sup>2</sup>, M. Noll<sup>1</sup>

<sup>1</sup>Coburg University of Applied Sciences, Institute for Bioanalysis, Coburg, Germany

<sup>2</sup>Bavarian Health and Food Safety Authority (LGL), National reference center for borrelia, Munich, Germany

Various microbial pathogens have been found in *Ixodes ricinus*, a common tick in central Europe. However, most studies assess microbial communities of ticks without prior decontamination of the tick skin surface, which may alter the results and mislead conclusions regarding the tick microbiome. The aim of this study was to test four different skin decontamination methods namely i.) 70% Ethanol, ii.) DNA away, iii.) 5 % sodium hypochlorite and iv.) Reactive-skin-decontamination-solution (RSDL) that were previously reported for tick, surface, animal or human skin decontamination. To test efficiency of decontamination, we contaminated each tick with a defined mixture of *Escherichia coli*, *Micrococcus luteus*, *Pseudomonas fluorescens*, *Cocksfoot mild mosaic virus* (CfMMoV), dog saliva and human sweat. As control no contamination or no decontamination strategy was carried out. After DNA- and RNA-extraction the recovery rate of contaminants was determined by qPCR and tick-borne bacterial community analyses by 16S rRNA gene amplicon sequencing. QPCR results showed that 5% sodium hypochlorite is the best decontamination strategy followed by DNA away, RSDL and 70% ethanol. Correspondence analyses (CA) of the bacterial community composition confirmed our qPCR results. Moreover, bacterial community composition of ticks decontaminated by 5% sodium hypochlorite clustered with negative controls in CA, indicating that removal of tick contaminants was superior. In contrast, common decontamination by 70% ethanol were less efficient. Decontamination of outer skin microbiome from ticks is essential to retrieve unbiased tick microbiome and to detect its pathogens.

---

### 321-MSHP

Targeted design and manipulation of defined microbial consortia by bacteriophage

\*A. von Strempel<sup>1</sup>, J. Wittman<sup>2</sup>, B. Stecher-Letsch<sup>1</sup>

<sup>1</sup>Max von Pettenkofer-Institut, München, Germany

<sup>2</sup>DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany

The human gut is a complex and diverse ecosystem, consisting of eukaryotic cells, bacteria and viruses. Bacteriophages are the most abundant viruses in the gut and yet one of the least understood. Through our modern diet and drugs like antibiotics, changes in the microbial and viral communities can occur which are associated with diseases like inflammatory bowel disease or gut cancer.

To obtain functional insights, we aim to investigate the interaction of bacteriophages and cognate host bacteria in the mammalian gut. Therefore, we isolated specific phages from environmental samples for one member of a minimal bacterial consortium, the Oligo-MM12, which consists of 12 well-characterized bacterial strains that colonize gnotobiotic mice in a stable and reproducible manner. Phages were characterized by genome sequencing and in terms of host-specificity and infection kinetics on the cognate host bacteria. In future studies, we aim to analyze the dynamic effect of phages on the stable bacterial community in the murine gut with respect to compositional, transcriptomic and metabolomic changes. Our work will yield insights into the ecological importance of phages in the gut, which will be important for evaluating the future use of phages for targeted microbiome manipulation.

### 322-MSHP

Influence of non-antibiotic drugs on EHEC growth and Shiga toxin production

\*B. von Armanseperg<sup>1,2</sup>, M. Koeppl<sup>1,2</sup>, J. Glaser<sup>1</sup>, A. Typas<sup>3</sup>, B. Stecher-Letsch<sup>1,2</sup>

<sup>1</sup>Max von Pettenkofer-Institute, Hygiene and Medical Microbiology, Faculty of Medicine, LMU Munich, München, Germany

<sup>2</sup>German Center of Infection Research (DZIF), Partner Site Munich, München, Germany

<sup>3</sup>European Molecular Biology Laboratory, Heidelberg, Germany

Enterohemorrhagic *Escherichia coli* (EHEC) is a food-borne human enteric pathogen that causes haemorrhagic colitis. A fraction of infected patients develop haemolytic uremic syndrome (HUS), which is potentially fatal. HUS is mediated by bacterial production of Shiga toxins (Stx) in the gut. A generally accepted causal therapy of haemorrhagic colitis in order to prevent the development of HUS is missing. Since recent outbreaks concur with the appearance of more virulent strains, there is a great need to explore novel treatment strategies.

Stx is an AB toxin encoded on lambdoid prophages. The toxin is induced by the bacterial SOS response and expressed during the phage lytic cycle. Besides DNA-damaging antibacterials, little is known on environmental risk factors promoting Stx production in the human gut. Here we aim to assess the effect of non-antibiotic drugs on EHEC growth and induction of Shiga toxin production, as they are known to substantially affect bacterial growth and physiology.

For screening, we use a reporter strain expressing *Gussia luciferase (gluc)* instead of *stx-2 (E.coli C600W34 stx2A::gluc aphT)*. This reporter is unique as it emulates both, Stx-2 production in the bacteria and the release into the environment by phage lysis. This reporter system is employed to screen a 1,200-component library for drugs inhibiting or promoting Stx-2 release by EHEC.

Based on these data we envision to identify drugs promoting Stx production during EHEC infection, which may therefore increase the risk of HUS development in infected patients. On the other hand, we aim to discover drugs inhibiting Stx production, which can be repurposed to prevent HUS development in infected patients.

### 323-MSHP

*Dyadobacter* sp. HH091 stimulates microalga growth and photosynthesis

\*Y. Astafeyeva<sup>1,2</sup>, D. Hanelt<sup>2</sup>, W. R. Streit<sup>1</sup>, I. Krohn<sup>1</sup>

<sup>1</sup>Institute of Plant Science and Microbiology/Universität Hamburg, Microbiology & Biotechnology, Hamburg, Germany

<sup>2</sup>Institute of Plant Science and Microbiology/Universität Hamburg, Aquatic Ecophysiology and Phycology, Hamburg, Germany

Microalgae are of high relevance for the global carbon cycling and it is well-known that they are associated with a diverse microbiota. However, it remains not well defined if the associated microbiota is specific for the microalga and which role individual bacterial taxa play. Further it is not understood which signals are involved in this complex interaction.

Our goal is to identify key mechanisms and signals involved in the tight interaction of bacteria and microalga. Therefore, we sequenced the microbiomes of the different alga and isolated various bacterial strains affiliated with the alga. In addition, we established synthetic plant-bacteria systems of various microalgae and bacteria isolates. One of the obtained isolates, HH091, which is affiliated with the phylum Bacteroidetes and the genus *Dyadobacter* strongly

stimulated growth and photosynthesis of the alga when it was added to "axenic" cultures. To further advance, we are currently studying the interaction of the isolate HH091 with the microalga *Scenedesmus acuminatus* SAG 38.81 and *Micrasterias radians* MZCH 672. For this, the whole-genome of the strain HH091 and the two microalga were established. The bacterial genome had a size of 7.8 Mb and the alga had sizes of approximately 119 Mb. The genome of HH091 contains multiple quorum sensing (QS) and quenching mechanisms possibly involved in the alga bacteria interaction. Current work focusses on characterizing the main QS signaling compounds.

### 324-MSHP

Rearing strategies in the black soldier fly influence microbial community composition and antibiotic/detergent-resistance gene abundance in the gut and feed residue

\*Y. A. Cifuentes Triana<sup>1</sup>, S. P. Glaeser<sup>1</sup>, J. Mvie<sup>1</sup>, J. O. Bartz<sup>1</sup>, A.

Müller<sup>2</sup>, H. Gutzeit<sup>2</sup>, A. Vilcinskas<sup>1,3</sup>, P. Kämpfer<sup>1</sup>

<sup>1</sup>Justus-Liebig-Universität Gießen, Gießen, Germany

<sup>2</sup>Technische Universität Dresden, Dresden, Germany

<sup>3</sup>Fraunhofer Institute for Molecular Biology and Applied Ecology, Gießen, Germany

*Hermetia illucens*, commonly known as black soldier fly (BSF), is one of the most promising insects for future application to recycle organic waste in combination with the production of high quality proteins as feed for agricultural animals. The gut microbiota plays a key role in the digestion efficiency and the deactivation of potential pathogenic bacteria of the organic waste used as substrate. We aimed to determine the bacterial core microbiota of BSF larvae; investigated changes in the bacterial community diversity and composition present in feed residues during BSF growth; and studied changes in the presence and/or transfer of antibiotic/disinfectant-resistance genes among the gut and residue microbiota. BSFs were reared in chicken feed. At three rearing stages the gut and residual feed microbiota were investigated in parallel by 16S rRNA gene amplicon based Illumina MiSeq sequencing. Antimicrobial resistance and disinfectant genes were quantifying using realtime PCR assays. Non-metric multidimensional scaling of bacterial community patterns indicated that the gut microbiota and the residue-associated bacterial communities were clearly different among each other. Both changed over time. Significant changes of the bacterial diversity and community compositions were only observed in the residue. Many representatives of bacterial genera containing potential pathogens, as *Staphylococcus* spp. or *Acinetobacter* spp., strongly decreased in relative abundance in the residue during rearing. Neither antibiotic nor disinfectant resistance genes accumulated in larvae but the abundance of *qacE* genes significantly increased in the residue of the given substrate. Rearing of BSF larvae has a strong impact on the bacterial community in the feed residue. The gut microbiota remain more stable with a set of abundant taxa that are persistent throughout the larval development and may be considered as the BSF gut core microbiome. The prevalence of antibiotic/disinfectant-resistance genes indicates, that this is another factor that must be evaluated carefully.

### 325-MSHP

*Lactobacilli* boost bioavailability of polyphenols in onion

\*M. Dormeyer<sup>1</sup>, L. Garbe<sup>1</sup>, A. Brauner<sup>1</sup>, D. Guyonnet<sup>2</sup>, R. Wittlake<sup>3</sup>, C. Koch<sup>1</sup>

<sup>1</sup>Symrise AG, CI - Microbiology Research, Holzminden, Germany

<sup>2</sup>Diana Nova, Paris, France

<sup>3</sup>Symrise AG, Analytical Research Center, Holzminden, Germany



**Introduction:** Traditional fermentation procedures as Natto from Japan and Sauerteig from Germany have been conserved throughout continents and centuries. Using bacteria to improve the healthy impact of food is anchored in most cultures. This passes out of mind in the young western world, where many people are suffering from different types of inflammation in the small intestine like the irritable bowel syndrome. The best help is a change of nutrition to a high-fiber diet including the uptake of probiotics as present in fermented products.

**Objective:** Onion peels contain fiber and are a great source of healthy polyphenols as quercetin. Unfortunately, quercetin is not bioavailable in its glycosylated form. Our idea is to find a probiotic *Lactobacillus* strain to degrade the sugar residues and make the quercetin bioavailable.

**Methods:** A quantitative screening was established ranking the *Lactobacilli* according to their abilities to degrade sugars. Afterwards onions were fermented and analyzed using the best candidates. The effect of bioavailable quercetin was investigated in an inflammation assay using human intestinal cells. To test common probiotic characteristics, we mimicked stomach transition by an *in vitro* stomach assay and examined the antimicrobial impact against common gut pathogens.

**Results:** *Lactobacilli* have very different metabolic abilities reflected in the varying emergence of quercetin in the fermented onion extract. This quercetin, but not quercetin-derivatives, is able to reduce inflammation caused by *Escherichia coli* in intestinal cells. The *in vitro* stomach passage assay reveals increased survival for some *Lactobacilli* correlating with their ecological niche. Furthermore, we show that *E. coli* and *Salmonella enteritidis* cannot establish growth in the presence of certain *Lactobacilli*.

**Conclusion:** Our research shows the great variation of species across the genus *Lactobacillus* in terms of sugar metabolism, acid tolerance, and interaction with both onion extracts and intestinal pathogens. The overall workflow turned out to be well suited to test probiotic bacteria with vegetable extracts and develop the best combinations for successful fermentation.

---

### 326-MSHP

A multi-omics approach for fecal swine microbiomes reveals insights into structural and functional dynamics during viral infection.

\*A. Meene<sup>1</sup>, L. C. Gierse<sup>1</sup>, D. Schultz<sup>2</sup>, H. Wang<sup>1</sup>, T. Schwaiger<sup>3</sup>, C. Schröder<sup>3</sup>, M. Lalk<sup>2</sup>, K. Riedel<sup>1</sup>, T. Urich<sup>1</sup>

<sup>1</sup>University of Greifswald, Institute of microbiology, Greifswald, Germany

<sup>2</sup>University of Greifswald, Institute for Biochemistry, Greifswald, Germany

<sup>3</sup>Friedrich-Loeffler Institute, Isle of Riems, Department of Experimental Animal Facilities and Biorisk Management, Greifswald, Isle of Riems, Germany

#### Introduction

Having access to appropriate pathogenicity models is crucial to investigate host-pathogen interactions during bacto-viral co-infections, which are characterized by their high mortality. Due to its large accordance to humans regarding its physiology, intestinal commensals and its immune system, swine has become an attractive alternative. Recent studies indicate a clear correlation between structure and function of

the human microbiota, progression of infections and consequently also the host health status.

#### Objectives

The aim of this study was to establish a high throughput multi-omics method for homogenization of heterogeneous fecal samples for data-integration of 16S rRNA gene-sequencing with metaproteome and metabolome datasets to get new insights into host-microbiome relationship within this complex environment which had been remained hidden when targeting only one single molecule.

#### Methods

Deep frozen homogenization protocol opened an integrated sample preparation for different Omics-techniques out of one sample. Nucleic-acid extractions from fecal powder and intestinal samples were performed via phenol-chloroform extraction followed by state-of-the-art 16S rRNA gene-sequencing and bioinformatic processing in R.

#### Results

Targeting the 16S rRNA gene reveals highly stable intestinal community compositions in healthy swine with reproducible successions in relevant families e.g. *Lactobacillaceae* and *Streptococcaceae*. These longitudinal shifts were also detected in the metaproteome data. Furthermore, we tested the possibility of using fecal samples as proxy for the intestinal status and observed a good accordance of fecal microbiota with the one from the proximal and distal colon.

#### Conclusions

The robust multi-omics pipeline enables a high throughput sample preparation with highly reproducible community composition in different target molecules. The opportunity of taking feces as proxy for the large intestine could pave the way for simple predictions of what is going on in the intestine with "minimal-invasive" sampling techniques and enables to screen a longer period of time without taking animals out of the experiment by dissection.

---

### 327-MSHP

Microbial community composition in microalgae cultures infected with aphelid parasites

\*A. L. Höger<sup>1</sup>, C. Griehl<sup>1</sup>, M. Noll<sup>2</sup>

<sup>1</sup>University of Applied Science Anhalt, Department of Applied Biosciences and Process Engineering, Koethen, Germany

<sup>2</sup>University of Applied Sciences and Arts, Institute for Bioanalysis, Coburg, Germany

The destruction of microalgae cultures in industrial production plants caused by biological contaminations represents a serious obstacle for the commercial use of algal biomass. Especially fungal infections with intracellular zoosporic parasites like Aphelida (*Ophistosporidia*) can lead to massive algae biomass loss. While contamination management is mainly based on fungicides, a preventive alternative approach to circumvent infections is the support of indigenous microbial communities of microalgae cultures.

The aim of this work is to investigate aphelid infection patterns on monocultures of several microalgae species commonly used in biotechnology. Microbial communities of microalgal cultures were retrieved from axenic or open industrial microalgae settings. Controlled contamination

experiments revealed a broad host specificity within the Chlorophyta displaying a decreasing degree of the aphelid infection with increasing phylogenetic distance to the main host. Beyond host species, composition of bacterial communities derived from open settings had a considerable impact on the development of the aphelid infection. Unialgal cultures harboured a highly diverse, algae specific indigenous microbiome. Bacterial community composition changed in aphelid presence over time, while it remains stable against the exposure to the open environment.

In conclusion, results indicate that complex microalgal, bacterial and fungal community compositions are affecting the patterns of aphelid infection. Findings will help to achieve a more stable microalgal production in industrial photobioreactors.

### 328-MSHP

The lichen microbiome and its role in adaptation to climate associated factors

\*M. Grimm<sup>1</sup>, T. Kroniger<sup>1</sup>, U. Schiefelbein<sup>2</sup>, D. Zühlke<sup>1</sup>, J. Bernhardt<sup>1</sup>, K. Riedel<sup>1</sup>

<sup>1</sup>University of Greifswald, Institute of Microbiology, Greifswald, Germany

<sup>2</sup>Blücherstraße 71, Rostock, Germany

Lichens, symbiotic organisms composed of a myco- and photobiont, represent a frequent and successful lifestyle on earth. In comparison to other lichens, *Lobaria pulmonaria* L. Hoffm. is less stress-resistant. It only tolerates a quite narrow range of ecological conditions and prefers relatively cool and humid habitats with hardly any air pollution. Our ongoing project aims on deciphering genotypic and phenotypic traits enabling this model lichen to adapt to changing environmental and especially climate conditions.

Within the last decade, bacterial communities were identified as surprisingly abundant, stable, specific, and structurally integrated elements of the classical lichen symbiosis. The diverse microbiota seems to contribute multiple aspects to the symbiotic system, including essential functions such as nutrient supply, pathogen defense, resistance against abiotic factors or support of fungal and algal growth. We hypothesize that especially changes in the composition and functionality of the lichen microbiome may enable the holobiont to cope with environmental changes and, thus, can be considered as an important ecological trait mediating lichen plasticity.

To strengthen our hypothesis we performed state-of-the-art metaproteome analyses of 15 lichen samples which have been collected along gradients of varying environmental factors such as high/less precipitation, maritime/alpine influence as well as maritime/continental climate. In general, Alphaproteobacteria, Gammaproteobacteria, Actinobacteria and Firmicutes seem to dominate the bacterial lichen microbiome that remains constant to a certain degree but shows some variations regarding the sampling sites. In a first attempt, we looked for correlations in the microbiota's metaproteome composition or the lichen phenotype with the changing environment. To approve these findings, a reciprocal transplantation experiment with samples from two different sites is currently under investigation.

### 329-MSHP

Investigating the mode of action of Bisphenols on the simplified human intestinal microbiota (SIHUMIx)

\*S. Riesbeck<sup>1</sup>, S. S. Schäpe<sup>1</sup>, J. L. Krause<sup>2</sup>, R. K. Masanetz<sup>1</sup>, U. Rolle-Kampczyk<sup>1</sup>, C. Eberlein<sup>3</sup>, H. J. Heipieper<sup>3</sup>, G. Herberth<sup>2</sup>, H. Petruschke<sup>1</sup>, N. Jehmlich<sup>1</sup>, M. von Bergen<sup>4,1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Molecular Systems Biology, Leipzig, Germany

<sup>2</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Immunology, Leipzig, Germany

<sup>3</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Biotechnology, Leipzig, Germany

<sup>4</sup>University of Leipzig, Institute of Biochemistry, Faculty of Biosciences, Pharmacy and Psychology, Leipzig, Germany

Bisphenols are industrial chemicals used in the process of polymerization of polycarbonate plastics and epoxy resins. Based on the variety of plastic products used in our everyday life, we are exposed to Bisphenols through the skin or diet. After banning Bisphenol A (BPA) in several products, Bisphenol S (BPS) and Bisphenol F (BPF) are widely used as alternatives. The microbial disrupting effect of BPS to intestinal microbial communities has already been reported, but less is known on how BPS affects bacterial activity and function. To analyze effects of BPS, we cultivated the simplified human gut model community (SIHUMIx, composed of 8 species) in bioreactors in order to determine the impact of BPS exposure (45 µM) on the community structure and function. By determining biomass and applying targeted metabolomics and metaproteomics, it was shown that biomass and short-chain fatty acid (SCFA) production were unaffected. Functional analysis revealed increased lipid, phospholipid and glycan metabolism in BPS exposed SIHUMIx. *E. coli* was identified to be affected in lipid biosynthesis whereas *B. thetaiotaomicron* is affected in phospholipid metabolism. To investigate membrane fluidity, fatty acid methyl esters (FAMES) profiles were compared. Changes in individual membrane fatty acid composition could not be clearly described; however the saturation level of the membranes slightly increased during BPS exposure. So far, this is the first study revealing functional changes of intestinal bacteria after BPS exposure when cultivated in continuous bioreactors. In addition, the effects of BPF on a single strain of SIHUMIx were investigated to identify the mode of action. Analyses will be performed regarding growth, targeted metabolomics and proteomics. This will give a closer insight into the effect mechanism of bisphenols, used as BPA alternative, onto the human microbiome.

### 330-MSHP

Gut microbiota in Foxp3<sup>+</sup> regulatory T cell depleted mice

\*J. Kehrmann<sup>1</sup>, L. Effenberg<sup>1</sup>, C. Wilk<sup>1</sup>, D. Schoemer<sup>1</sup>, P. Nhi Ngo Thi<sup>1</sup>, A. Adamczyk<sup>1</sup>, E. Pastille<sup>1</sup>, R. Scholtysik<sup>2</sup>, L. Klein-Hitpass<sup>3</sup>, R. Klopffleisch<sup>4</sup>, A. Westendorf<sup>1</sup>, J. Buer<sup>1</sup>

<sup>1</sup>University Hospital Essen, University of Duisburg-Essen, Institute of Medical Microbiology, Essen, Germany

<sup>2</sup>University of Duisburg Essen, Institute of Cell Biology (Cancer Research), Essen, Germany

<sup>3</sup>University of Duisburg Essen, Biochip Laboratory, Institute for Cell Biology - Tumor Research, Essen, Germany

<sup>4</sup>Freie Universitaet Berlin, Institute of Veterinary Pathology, Berlin, Germany

### Introduction

The intestine contains a high abundance of Foxp3<sup>+</sup> regulatory T (Treg) cells. They are fundamental for maintaining the intestinal homeostasis by controlling immune responses of the innate and adaptive immune system but their precise impact on the gut microbiota is unclear.

### Objective

We analysed the effects of Treg cell depletion on inflammation of the gut mucosa and studied gut microbiota

before and after Treg cell depletion using the DEREg (DEpletion of REGulatory T cells).

## Materials and methods

We extracted DNA from stool samples of DEREg mice and wildtype littermates at five different time points before and after Diphtheria Toxin (DT) application to deplete Treg cells in DEREg mice. We used the V3/V4 region of the 16S rRNA gene to study the gut microbiota with Illumina MiSeq paired ends sequencing.

## Results

The majority of gut microbiota samples from late time points after Treg cell depletion in DEREg mice was separated from samples of early time points before Treg cell depletion in these mice and from gut microbiota samples of wildtype mice by multidimensional scaling. Treg cell depletion in DEREg mice was followed by an increase in the relative abundance of the phylum Firmicutes and by intestinal inflammation in DEREg mice 20 days after Treg cell depletion. In addition, the variables cage, breeding and experiment number were associated with differences in the gut microbiota composition.

## Conclusions

Our study indicates that Treg cells influence the gut microbiota composition. Furthermore, the variables cage, breeding and experiment number should be respected in murine studies.

---

### 331-MSHP

Temporal dynamics of human intestinal microbiota and its implications for 16S-rRNA based analysis

N. Hillebrandt<sup>1</sup>, D. Graf<sup>1</sup>, \*A. Dötsch<sup>1</sup>

<sup>1</sup>Max Rubner-Institut, Department of Physiology and Biotechnology of Nutrition, Karlsruhe, Germany

**Introduction:** Analysing Intestinal Microbiota (IM) using stool samples and sequencing the 16S rRNA gene has become a standard application. Many factors affect the results, thus standardized methods are of major importance. However, efforts to standardize methods cannot reduce the amount of biological variation within any individual.

**Objectives:** To determine the temporal variation of IM composition, the IM of four individuals was observed for a period of 14 days.

**Methods:** Stool samples of 4 volunteers were collected during 14 days. DNA was isolated using the NucleoSpin Stool DNA kit (Macherey-Nagel) with Bead Beating on a FastPrep device. The V4 region of the 16S rRNA gene was amplified using dual index primers and sequenced on Illumina MiSeq with 2x300 bp. Amplicon sequences were analysed with mothur using the SILVA SSU database and further evaluated with the R package phyloseq.

**Results:** The IM of all four individuals showed a typical composition dominated by Firmicutes, Bacteroidetes and Actinobacteria and each individual's IM composition was clearly different from the others. Most of the detected bacterial genera were present in all four IM but there were also several genera specific to individual microbiotas. Variation of the IM within one individual over time was smaller in comparison to the inter-individual variation but still

showing large differences from day to day. All bacterial genera were affected but none disappeared completely or occurred for the first time within the two weeks. Local variation between samples collected from the same faeces was also analysed and found to be of similar magnitude compared to the temporal variation. The results highlight the complex spatiotemporal dynamics of faecal microbiome measurements that are commonly used for IM analysis.

**Conclusion:** The healthy human intestinal microbiota is in a state of dynamic equilibrium that varies around a steady state from day to day and also locally within individual stools samples. Studies focusing on microbiota analysis should therefore use sample homogenization, multiple replicates or sufficiently large group sizes to mitigate the effect of this "biological noise".

---

### 332-MSHP

**Towards high-resolution metagenomics to unravel gut microbiome functionality of solitary bees**

\*Y. Pinheiro Alves de Souza<sup>1,2</sup>, A. Keller<sup>3</sup>, M. Schlöter<sup>1,4</sup>, B. Foesel<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München, COMI, Oberschleißheim, Germany

<sup>2</sup>Technical University of Munich, Lehrstuhl für Bodenkunde, Freising, Germany

<sup>3</sup>University of Würzburg, Department of Bioinformatics, Würzburg, Germany

<sup>4</sup>Technical University of Munich, Central Institute for Food and Health (ZIEL), Freising, Germany

The worldwide decline of bees is not only an issue of biodiversity


conservation, but strongly affects human food supply, since up to 70% of pollination is done by bees. Solitary bees, which can reach pollination rates up to 100 x higher than honey bees, are considered as key pollinators for some plant species, but the link between the gut microbiome and their health remains poorly studied. Previous data show that changes in the gut microbiome composition (caused by factors like land use, fertilization and flower diversity) make bees more prone to infection by opportunistic pathogens. However, most of the recent studies have used next

generation sequencing (mainly 16S rRNA barcoding) to understand the impact of environmental conditions on the intestinal microbiome of bees, but there is still a lack of information on major functional traits of the gut microbiome of bees and genes which trigger host – microbe interactions in the gut.

The aim of this study was to optimize the pipeline for metagenomic library construction, by addressing issues like minimum number of individuals needed, optimization of DNA extraction, minimum DNA amount for a functional metagenomic library building, host DNA depletion, and Multiple Displacement Amplification (MDA) bias using guts from

commercially acquired *Osmia bicornis* pupae. Our result indicate that the microbial communities are well represented with minor differences introduced by the DNA extraction procedure, with Alphaproteobacteria (21%),

Actinobacteria (21%) representing the most abundant phyla which is in accordance with recent 16S rRNA gene based barcoding data. Host-DNA depletion using the methylation of the host DNA as a target, did not result in a significant reduction in host DNA, indicating low methylation levels of the host DNA. Using MDA based pre-amplification, DNA amounts of less than 0,1 ng resulted in DNA libraries which could be used for the generation of metagenomes. However MDA introduced a significant shifts in the microbiome structure mainly when DNA amounts

less than 10 ng were used for amplification, with an increase in relative abundance of Bacilli and Betaproteobacteria. 

### 333-MSHP

Can bacteria adapted to leaf defense metabolites influence the secondary colonization of *Arabidopsis thaliana* leaves?

\*K. Unger<sup>1</sup>, P. Baumann<sup>1</sup>, T. Mayer<sup>1</sup>, M. Reichelt<sup>2</sup>, J. Gershenzon<sup>2</sup>, M. Agler<sup>1</sup>

<sup>1</sup>Friedrich-Schiller-University, Plant Microbiosis Lab - Department of Microbiology, Jena, Germany

<sup>2</sup>Max Planck Institute for Chemical Ecology, Department of Biochemistry, Jena, Germany

#### Introduction

Secondary metabolites are critical for defense in most plants. A well understood example is the glucosinolate-myrosinase system in *Brassicaceae* which plays roles in drought stress, defense against herbivores and microbial pathogens. Isothiocyanates, the hydrolysis products of glucosinolates, are important in terms of non-host resistance, by contributing antimicrobial activity against some plant pathogens. In turn, several colonizers of *A. thaliana* express *sax* genes that confer tolerance to isothiocyanates, in part by degrading them.

#### Objectives

We hypothesize that well-adapted members of the leaf microbiome influence the success of secondary colonization by detoxifying the environment. We aim to evaluate the role of toxic isothiocyanate compounds in this process.

#### Materials & methods

We isolated and characterized aliphatic glucosinolates in five *A. thaliana* populations from Jena, Germany. To determine whether and to what extent leaves select colonizers, we used a high-throughput gnotobiotic plant system to enrich leaf-adapted bacteria and compared their diversity to media isolates. To recover species adapted to specific plant chemistries, we are performing cross-enrichments of microorganisms from two plant populations. Using plant mutants and a metabolomics approach, we aim to characterize resistance to isothiocyanates and evaluate effects on secondary colonization and pathogen invasion.

#### Results

Plant isolates from the five populations differed strongly in terms of 14 aliphatic glucosinolates. *In-planta* enrichments revealed that although there is a plethora of culturable diversity on leaves, only isolates from a few major genera (*Pseudomonas*, *Xanthomonas*, *Rhizobium*) can efficiently colonize leaves alone. Ongoing experiments are evaluating resistance to glucosinolate-derived toxins in these isolates and how these resistance mechanisms affect secondary colonization.

#### Conclusion

Our results support the hypothesis that plant hosts strongly select for well-adapted microbes that in turn pave the way for secondary colonization of leaves.

### 334-MSHP

Influence of gut commensal bacteria on intestinal barrier function

\*N. Mohebbi<sup>1</sup>, B. Kreikemeyer<sup>1</sup>, A. Breitrück<sup>1,2</sup>

<sup>1</sup>Institute of Medical Microbiology, Virology and Hygiene University Medicine Rostock, Rostock, Germany

<sup>2</sup>Fraunhofer Institut für Zelltherapie und Immunologie - IZI, Extrakorporale Immunmodulation (EXIM), Rostock, Germany

#### Introduction:

Chronic intestinal inflammation disrupts intestinal barrier function and may contribute to the pathology of gastrointestinal disorder as seen in inflammatory bowel disease (IBD). There is evidence that gut microbiota regulate intestinal barrier function and may contribute to the protective mechanism of the intestinal mucosa against the development of chronic inflammation.

#### Objective:

In this study we investigate the protective effect of three anaerobic commensal bacterial species of *Faecalibacterium prausnitzii*, *Roseburia intestinalis* and *Bacteroides faecis* independently and in combination on intestinal barrier function and intestinal inflammation.

#### Methods:

Differentiated Caco-2 and HT29-MTX cells on transwell inserts were used as a model of intestinal barrier permeability. The barrier integrity of cells was assessed by measuring transepithelial electrical resistance (TEER) and flux of 4 kDa fluorescein isothiocyanate-dextran (FD) after incubation with or without the commensal bacteria. In the next step to mimic IBD conditions, differentiated cells were treated with a pro-inflammatory stimulus and incubated with commensal bacteria. The expression level of pro-inflammatory cytokines was measured by multiplex bead ELISA assay.

#### Results:

Experimental results indicated that all three species positively affected the barrier function of differentiated Caco-2 and HT29-MTX cells. The pro-inflammatory decreased TEER value was significantly increased after incubation of cells with bacteria. The same trend was observed for co-administration of all three strains. Significant decrease in FITC-labeled dextran flux of inflamed epithelial cells after incubation with bacteria supported the TEER result. Pro-inflammatory cytokines levels were attenuated compared to cells that had not been treated with commensal bacteria.

#### Conclusions:

In conclusion all three examined commensal bacterial species were found to have a high potential for re-establishment and curing of intestinal barrier function in IBDs condition. The therapeutic and prophylactic potential of our commensal species is currently assessed in an experimental colitis animal model.

### 335-MSHP

Evaluation of the T6SS-associated activities of four VgrG proteins in *Aeromonas veronii* Hm21

\*J. Marden<sup>1</sup>, J. Graf<sup>1</sup>, S. Sampath<sup>2</sup>

<sup>1</sup>University of Connecticut, Molecular and Cellular Biology, Storrs, United States

<sup>2</sup>Boston University School of Medicine, Boston, United States

Many *Aeromonas* species are beneficial symbionts of the medicinal leech and zebrafish, while others are pathogens of humans, fish and other animals. The type 6 secretion system (T6SS) has emerged as an important effector-delivery system that is involved in both bacterial and eukaryotic interactions. In a previous screen for *Aeromonas veronii* Hm21 factors important for colonization of the medicinal leech gut, we identified a mutant exhibiting a >100-fold defect in colonization compared to the wild-type strain that was subsequently shown to have a Tn5 insertion within a rearrangement hotspot (Rhs) protein-encoding gene. Interestingly, further examination of the Hm21 genome revealed the presence of four gene clusters encoding T6SS-associated proteins, each of which harbors a gene encoding a valine-glycine repeat protein G (VgrG) protein, an important structural component of the T6SS. We created single and combinatorial gene deletions of these VgrG-encoding genes to study their roles in promoting the T6SS-mediated killing of *A. veronii* Hm221, which does not contain a T6SS. Our results show that deletion of *vgrG2* reduces, but does not eliminate, the ability of *A. veronii* Hm21 to kill *A. veronii* Hm221. Furthermore, the deletion of *vgrG2* in combination with any of the three other *vgrG* genes completely abrogates the ability of *A. veronii* Hm21 to kill *A. veronii* Hm221, as does the loss of *vasK*, an essential component of the T6SS apparatus. These results demonstrate the importance of the *A. veronii* Hm21 T6SS and of specific VgrG proteins in mediating interbacterial interactions. Additional investigations are needed to elucidate whether the observed loss of killing is due to the loss of effectors that are specific to each VgrG protein or the inability of certain combinations of VgrGs to form a functional trimer.

### 336-MSHP

Investigations of the clonal relationship of *Actinomyces* strains in periodontal pockets at different depths using Diversilab, an automated repetitive sequenced based PCR bacterial typing system from BioMérieux

\*C. S. Stingu<sup>1</sup>, A. C. Rodloff<sup>1</sup>, H. Jentsch<sup>1,2</sup>, P. Vielkind<sup>1</sup>, T. Borgman<sup>1</sup>, L. Lehmeier<sup>1</sup>

<sup>1</sup>Institut für Medical Microbiology and Epidemiology of infectious Diseases, Leipzig, Germany

<sup>2</sup>Centre of Periodontology, Department for Cariology, Endodontology and Periodontology, University Hospital, Leipzig, Germany

*Actinomyces naeslundii* and *A. oris* are probably the most important dental plaque formers among the members of the *Actinomyces* spp. being involved in pathogenesis of different forms of periodontitis. Most of the previous studies usually determined the composition of the biofilm up to the species level, while the clonal diversity of individual strains being ignored.

The aim of the study was to find out the clonal relationship of *A. naeslundii* and *A. oris* strains in different depths of periodontal pockets of patients with chronic periodontitis.

#### Methods

A total of 223 *Actinomyces* clinical strains from 20 patients with periodontitis (former chronic periodontitis, new classification: Stadium 2 -3, Grad B) were used in this study. The strains were cultivated from supra- and subgingival biofilm of teeth with shallow pockets, deep pockets and very deep pockets. All the strains were identified using MALDI-TOF-MS method and by sequence analysis of the 16S ribosomal RNA gene. Rep-PCR typing of the cultivated strains was performed using DiversiLab 3.6.0.39.

The data were analysed using the Pearson's Correlation. We used the following similarity rules:

1. strains that showed < 95% similarity were classified as unrelated
2. strains that showed a similarity between 95%-98% were classified as closely related isolates
3. strains that showed > 98% similarity were classified as identical isolates

#### Results

Multiple colonization patterns were found. Seven patients were individually colonized with only unrelated isolates while 13 patients harbored both similar and unrelated isolates at different sites. The similar clones were found either supra-or subgingivally or both. Identical strains occurred in subgingival shallow depths more often than in other subgingival depths. The number of clones in one patient varied from 2 to 17 different Rep-PCR genotypes.

#### Conclusion

The clonal relationship between genotypes of *A. naeslundii* and *A. oris* in one individual is variable and unpredictable. It varies from the presence of multiple genotypes with no clonal similarity between them to the presence of only two different clones supra-or subgingivally at different sites.

### 337-MSHP

Arginase 1 promotes colitis due to L-arginine deprivation and intestinal dysbiosis

J. Baier<sup>1</sup>, M. Gänsbauer<sup>1</sup>, K. Dettmer<sup>2</sup>, A. Ekici<sup>3</sup>, W. Stefan<sup>4</sup>, C. Bogdan<sup>1</sup>, \*J. Mattner<sup>1</sup>

<sup>1</sup>Klinische Mikrobiologie Erlangen, Erlangen, Germany

<sup>2</sup>Institute for Functional Genomics, University of Regensburg, Regensburg, Germany

<sup>3</sup>Institute of Human Genetics, Erlangen, Germany

<sup>4</sup>Medical Clinic I, Erlangen, Germany

#### 1. Introduction

The metabolism of the semi-essential amino acid L-arginine in the alimentary tract is altered in animal models of colitis. Furthermore, the expression and activity of arginase 1 (Arg1) which utilizes L-arginine as substrate are enhanced in mucosal tissues of patients with inflammatory bowel disease (IBD).

#### 2. Aims

As dysbiosis is a signature of both, IBD patients and colitis models, we characterized the impact of L-arginine and Arg1 on intestinal microbiota and microenvironmental parameters, which mediate the induction, perpetuation and resolution of colitis.

#### 3. Material and Methods

We fed L-arginine-deprived and -supplemented chow to DSS-treated Tie2-Cre<sup>+</sup>/Arg1<sup>fl/fl</sup> mice lacking Arg1 in hematopoietic and endothelial cells and respective Arg1-expressing littermate controls. Whole-genome transcriptomic patterns, the intestinal metabolome and microbiome as well as the extent of intestinal inflammation were assessed using immunohistochemistry, high resolution endoscopy, qPCR, RNA sequence analyses, 16S rRNA sequencing, confocal laser scanning microscopy and HPLC.

## 4. Results

Tie2-Cre<sup>+</sup>/Arg1<sup>fl/fl</sup> mice recovered faster from experimental colitis than Arg1-expressing littermates. This unexpected observation correlated with compositional changes in the intestinal microbiota, altered cytokine and toll-like receptor expression patterns and an accumulation of intraluminal polyamines.

Arginine-free chow accelerated DSS-induced colitis while a supplementation of L-arginine ameliorated disease. Protection from disease was associated with an accumulation of *Christensenella* and *Ruminococcus* in L-arginine-supplemented wild-type litters, similar as observed in control chow fed Tie2-Cre<sup>+</sup>/Arg1<sup>fl/fl</sup> mice. Accordingly, fecal microbiota transfers (FMTs) from wild-type litters supplemented with L-Arginine restored the protective, anti-inflammatory phenotype in recipient mice similar as FMTs from control chow fed Tie2-Cre<sup>+</sup>/Arg1<sup>fl/fl</sup> donors.

## 5. Summary

Dietary L-arginine restriction abolished the protective effect of Arg1-deletion, suggesting that protection is related to an increased availability of L-arginine and the expansion of an anti-inflammatory microbiota.

### 338-MSHP

#### ***Akkermansia muciniphila* reduces bacterial colonization and intestinal inflammation by inhibiting the adhesion of *Salmonella* to epithelial cells**

\*A. Suwandi<sup>1,2</sup>, A. Galeev<sup>1,2</sup>, S. Sharma<sup>1,2</sup>, K. Seeger<sup>1,2</sup>, J. Braun<sup>3</sup>, D. Ring<sup>4,5</sup>, B. Stecher<sup>4,5</sup>, M. Blaut<sup>6</sup>, G. Loh<sup>6</sup>, G. A. Grassl<sup>1,2</sup>

<sup>1</sup>Hannover Medical School (MHH), Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany

<sup>2</sup>German Center for Infection Research (DZIF), Hannover, Germany

<sup>3</sup>CAU Kiel, Institute for Experimental Medicine, Kiel, Germany

<sup>4</sup>LMU Munich, Max-von-Pettenkofer Institute, Munich, Germany

<sup>5</sup>Germany Center for Infection Research (DZIF), Munich, Germany

<sup>6</sup>German Institute of Human Nutrition Potsdam-Rehbruecke, Gastrointestinal Microbiology, Nuthetal, Germany

Intestinal microbiota and the gastrointestinal mucus layer provide resistance against pathogens. *Akkermansia muciniphila* (AM) belonging to the *Verrucomicrobia* is specialized in the degradation of mucin. This anaerobic bacterium is found in the intestinal tract of humans and other mammals. *Salmonella enterica* serovar Typhimurium (S.Tm) is a food-borne pathogen and a major cause of gastroenteritis. To study the importance of AM presence to colonization resistance against S.Tm, gnotobiotic mice associated with a defined simplified human intestinal microbiota (SIHUMI) of eight bacterial species were utilized. These mice were complemented with or without AM, and then infected with S.Tm.

Interestingly, in the presence of AM, S.Tm caused significantly decreased histopathological changes as well as decreased levels of inflammatory cytokines such as TNF- $\alpha$  and IL-17 in the cecum of mice at day 14 after infection. In addition, lower bacterial counts in cecum and colon were observed in the group of mice with AM. Furthermore, *in vitro* experiments showed the presence of AM inhibited adhesion of *Salmonella* to differentiated, polarized HT29-MTX-E12 cells, but not to undifferentiated HT29-MTX-E12 cells. In addition, neither heat-killed AM nor cell-free culture supernatant of AM inhibited adhesion of S.Tm to differentiated HT29-MTX-E12 cells. These results indicate that the presence of live AM reduces S.Tm induced inflammation by inhibiting adhesion of *Salmonella*.

### 339-MSHP

Blood microbiota assessed by light and electron microscopy

\*S. Panaiotov<sup>1</sup>, E. Nikolova<sup>2</sup>, R. Kalfin<sup>3</sup>

<sup>1</sup>National Center of Infectious and Parasitic Diseases, Department of Microbiology, Sofia, Bulgaria

<sup>2</sup>Bulgarian Academy of Sciences, Institute of Experimental Morphology, Pathology and Anthropology, Sofia, Bulgaria

<sup>3</sup>Bulgarian Academy of Sciences, Institute of Neurobiology, Sofia, Bulgaria

## Introduction

Nevertheless, the blood microbiome is still an enigma, its existence in clinically healthy individuals was proven during the last 50 years. Indirect evidence from radiometric analysis suggested existence of life microbial forms in erythrocytes. More recently, targeted nucleic acids sequencing demonstrated rich microbial biodiversity of bacteria and fungi in the blood of clinically healthy individuals. There is lack of appropriate methods for cultivation and visualization. The morphology and proliferation cycle of normal blood microbiota *in vivo* and *in culture* are obscure.

## Objectives

To understand the life cycle of blood microbiota we focused on analysis by light and electron microscopy.

## Materials and Methods

Non-cultured whole blood and stress cultured lysed blood samples from healthy individuals were studied. Blood samples of healthy individuals were collected in K2 EDTA vacutainer tubes. Blood microbiota resuscitation was performed at 430C in BHI broth supplemented with vitamin K 1 mg/ml, 10% sucrose, 0.25% sodium citrate and 0.2% yeastolate. Samples were cultured for 24 hours. At every hour an aliquot was fixed in 2.5% glutaraldehyde. Preparations were observed under light and dark field microscopy, transmission and scanning electron microscopy.

## Results

We demonstrate that *in vivo* free circulating microbiota in whole blood possess a well defined cell wall and proliferate by budding or a mechanism similar to extrusion of progeny bodies by opening and closing the cell wall. By contrast, the blood microbiota *in vitro* stress culture proliferate as cell-wall deficient microbiota by forming electron-dense or electron-transparent bodies. During the first hour of cultivation dense elementary bodies in size of 0.2  $\mu$ m composed by thick cell coat were observed. The electron-dense bodies proliferate by fission or produce in chains Gram-negatively stained progeny cells or burst to release progeny cells. On the other site, the electron-transparent bodies enlarge and emit progeny cells through the membrane.

## Conclusions

The rich biodiversity of eukaryotic and prokaryotic microbiota identified in blood and our results suggest different *in vivo* and *in vitro* mechanisms of proliferation. Blood microbiota can reproduce in stress conditions by great variety of unusual modes, such as irregular binary fission, budding, protrusion-extrusion of elementary bodies. The observed morphological plasticity of blood microbiota might be due to the bacterial and fungi composition. A tentative hypothesis of the life cycles of the blood microbiota was developed.

**Acknowledgment:** This work was supported by grant DH-01-4/16.12.2016 of National Science Fund, Bulgaria.

\*L. Hahn<sup>1</sup>, C. Dahl<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Mikrobiologie und Biotechnologie, Bonn, Germany

### 340-MSHP

#### The influence of temperature and associated microbiota on the toxicity of the dinoflagellate *Prorocentrum minimum*

\*S. Sanchez Garcia<sup>1</sup>, J. Mansky<sup>1</sup>, H. Wang<sup>1</sup>, H. Sztajer<sup>1</sup>, J. Tomasch<sup>2</sup>, J. Leinberger<sup>3</sup>, T. Brinkhoff<sup>3</sup>, J. Holste<sup>4</sup>, S. Schulz<sup>4</sup>, \*I. Wagner-Döbler<sup>1</sup>

<sup>1</sup>Technische Universität Braunschweig, Microbiology, Braunschweig, Germany

<sup>2</sup>Helmholtz-Centre for Infection Biology (HZI), Molecular Bacteriology, Braunschweig, Germany

<sup>3</sup>University of Oldenburg, ICBM, Oldenburg, Germany

<sup>4</sup>Technical University of Braunschweig, Organic Chemistry, Braunschweig, Germany

The dinoflagellate *Prorocentrum minimum* is a ubiquitous coastal algae which causes harmful algal blooms and is suspected to produce tetrodotoxin like compounds at higher temperatures. It is an invasive species in the North Sea. We cultivated four non-axenic and one axenic isolate of *P. minimum* at 20°C, 26°C and 30°C and screened for toxicity using a neuroblastoma (Neuro-2) cell line in combination with HPLC-MS-MS. The microbiota composition of the non-axenic algae strains was determined using 16S rRNA gene amplicon sequencing. Our aim is to describe a possible core microbiota of *P. minimum*, observe the adaptation of the microbiota towards higher temperatures, and to identify toxins produced by *P. minimum* either alone or in co-culture with its associated bacteria.

### 341-MSHP

#### Membrane vesicles in an algae-bacteria co-culture

\*J. Mansky<sup>1</sup>, S. Sanchez Garcia<sup>1</sup>, H. Wang<sup>1</sup>, H. Sztajer<sup>1</sup>, L. Woehlbrand<sup>2</sup>, R. Rabus<sup>2</sup>, M. Neumann-Schaal<sup>3</sup>, P. Henke<sup>3</sup>, J. Overmann<sup>3</sup>, J. Tomasch<sup>4</sup>, D. Koteska<sup>5</sup>, S. Schulz<sup>5</sup>, I. Wagner-Döbler<sup>1</sup>

<sup>1</sup>Technical University of Braunschweig, Microbiology, Braunschweig, Germany

<sup>2</sup>University of Oldenburg, ICBM, Oldenburg, Germany

<sup>3</sup>Leibniz Institute DSMZ, Braunschweig, Germany

<sup>4</sup>Helmholtz-Centre for Infection Research (HZI), Molecular Bacteriology, Braunschweig, Germany

<sup>5</sup>Technical University of Braunschweig, Organic Chemistry, Braunschweig, Germany

Membrane vesicles (MV) are an emerging mechanism of cell-cell communication. The marine alphaproteobacterium *Dinoroseobacter shibae* and the dinoflagellate *Prorocentrum minimum* form a so-called Jekyll-and-Hyde interaction, consisting of a mutualistic and a pathogenic phase, but both can also be grown in single culture. We isolated MVs from both organisms and determined their cargo and functions. Membrane vesicles of *D. shibae* are enriched for the terminus of chromosome replication, particularly the 22 bp recognition sequence of the XerCD recombinases, and proteins that interact with the septum prior to cell division. From the metabolome inside the vesicles, an aldehyde and a fatty acid were isolated and shown to have algicidal activity. *P. minimum* secretes ~315 vesicles per cell throughout growth. They contain unusual sulfo-lipids in their membranes.

### 342-OTP

#### Maturation of lipoate-binding proteins in microbial sulfur oxidation

Recently, an unexpected metabolic function was discovered for the well-known cofactor lipoic acid. Novel lipoate-binding proteins (LbpA) resembling classical glycine cleavage system H (GcvH) proteins are indispensable components of the new sulfur-oxidizing heterodisulfide reductase-like enzyme system (sHdr) that occurs in a large number of chemolithotrophic sulfur-oxidizing prokaryotes [1,2]. LbpA proteins are specifically designed for sulfur oxidation and cannot functionally replace GcvH [2,3]. Usually, *shdr-lbpA* genes are linked with genes for enzymes typically involved in maturation of lipoate-binding proteins, including lipoate-protein ligases and two different radical SAM domain proteins. This is surprising because all *shdr*-containing sulfur oxidizers contain at least one of the established lipoate maturation pathways, i.e. lipoate scavenging or *de novo* synthesis involving the radical SAM domain protein LipA. We posed the question whether maturation of LbpAs from sulfur oxidizers follows a dedicated pathway.

Complementation studies revealed that sulfur oxidizer LbpA proteins do not serve as substrates for the lipoate-binding protein maturation machineries in *B. subtilis* and *E. coli* [2]. However, when provided with helper plasmids carrying LbpA maturation genes from sulfur oxidizers, *E. coli* produces modified LbpAs as shown by gel mobility shift assays. Mass spectrometry revealed that the modified LbpAs carried either octanoate or an octanoyl residue with only one instead of the two sulfur atoms in lipoate. Formation of such monothiolated forms is unprecedented. All sulfur-inserting LipA proteins characterized so far insert two sulfur atoms in one single step [4]. Complete lipoylation of LbpAs was achieved *in vitro* in the presence of lipoic acid and ATP catalyzed by recombinant lipoate-protein ligase (LplA) from sulfur oxidizers. This enzyme acts exclusively on LbpA proteins from sulfur oxidizers [2]. In conclusion, holo-LbpAs in sulfur oxidizers mature along a specific pathway that includes novel intermediates.

[1] Koch, Dahl 2018 ISME J 12, 2479

[2] Cao et al 2018 eLife 7, e37439

[3] Cao et al 2018 PNAS 115, 647

[4] Cronan 2016 Microb Mol Biol Rev 80, 429

### 343-OTP

#### Taxonomy-free dereplication of massive genome collections using embeddings of protein content

\*A. Viehweger<sup>1,2</sup>, M. Hölzer<sup>1,3</sup>, C. Brandt<sup>1,4</sup>

<sup>1</sup>nanozoo GmbH, Leipzig, Germany

<sup>2</sup>Leipzig University Hospital, Medical Microbiology and Epidemiology of Infectious Diseases, Leipzig, Germany

<sup>3</sup>Friedrich Schiller University Jena, RNA Bioinformatics and High-Throughput Analysis, Jena, Germany

<sup>4</sup>Jena University Hospital, Infectious Diseases and Infection Control, Jena, Germany

Question: Many recent microbial genome collections curate hundreds of thousands of genomes. This volume complicates many genomic analyses such as phylogeny reconstruction and taxon assignment because the computational hardware required to handle the sheer size of the collections is substantial. However, the number of representatives of each species is highly skewed towards human pathogens, and thus many genomes are superfluous

and could be removed. We aimed to create a resource-frugal genome dereplication method that could reduce massive genome collections based on genome sequence alone, without the need for manual curation nor prior taxonomic information.

**Methods:** We recently created a genome representation for bacteria and archaea called "nanotext". This method treats protein domains in a genome like words in a document and embeds each genome in a low-dimensional vector of numbers that captures known taxonomic relationships. Extending nanotext, we present a workflow called "thinspace" that employs unsupervised clustering of the nanotext vectors to group similar genomes. These groups are then dereplicated based on pairwise average nucleotide frequency.

**Results:** We were able to shrink a corpus of about 150 thousand genomes to about 22 thousand. Highly overrepresented species of human pathogens were reduced to only a handful of representative genomes. We compare this collection to a recently published one that has received extensive manual curation and is based on the *Genome Taxonomy Database*. Both collections perform nearly identical on the common task of read taxon assignment. Notably, both outperform the NCBI RefSeq collection, increasing the amount of assigned reads by multiples for a set of biogas and human stool samples.

**Conclusion:** With thinspace, massive genome collections can be dereplicated on regular hardware with similar quality compared to manually curated collections. The software is released under a BSD-3 license and is available at [github.com/phiweger/nanotext](https://github.com/phiweger/nanotext) and [github.com/phiweger/thinspace](https://github.com/phiweger/thinspace).

---

### 344-OTP

**BacDive:** Systematic Access to Phenotypic Data on 90% of the Bacterial Species

\*L. Reimer<sup>1</sup>, J. Sardà<sup>1</sup>, J. Overmann<sup>1</sup>

<sup>1</sup>Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany

Microbial phenotypic data are scattered throughout the scientific literature, databases and unpublished lab notes. However, hotspots for metadata can be identified for example internal files of microbial collections (1) or species descriptions in the literature (2).

(1) BacDive started in 2012 to collect data from internal files of microbial collections, standardize them and make them publicly available, starting with files from the DSMZ and private collections and then continuing to expand on other collections (e.g. CABI, CIP, CCUG). (2) In 2016 BacDive started to enlarge datasets with manually extracted information from species descriptions published in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM). So far already data from more than 6,500 species descriptions are accessible via BacDive.

Data in BacDive is collected in over 600 data fields covering taxonomy, morphology, physiology, cultivation conditions, origin, and molecular biology of the strains. The systematic access to these data enables queries like "show me all strains that are able to utilize a specific metabolite" by using the Advanced search or queries like "show me all strains isolated from a marine environment" by using the Isolation source search. With currently 9225 API® tests for 5320 strains, BacDive also offers the worldwide largest API® test

collection, which can be queried using the API test finder tool.

Up to now, BacDive offers data for more than 80,000 strains including over 14,000 type strains. Thereby the systematic access to phenotypic data of approx. 90% of the so far described prokaryotic biodiversity (based on 16,000 validly described species) is granted. The continued transformation of phenotypic data into a standardized format opens up new ways to analyze microbial diversity.

---

### 345-OTP

**New concepts in bacterial N-glycosylation – Substrate specificity of the EF-P arginine rhamnosyltransferase EarP**

\*R. Krafczyk<sup>1</sup>, F. Koller<sup>1</sup>, L. Lindenthal<sup>2</sup>, A. Sieber<sup>1</sup>, K. Jung<sup>1</sup>, J. Lassak<sup>1</sup>

<sup>1</sup>Ludwig-Maximilians Universität München, Mikrobiologie, Martinsried, Germany

<sup>2</sup>Max-Planck Institute, Biochemistry, Martinsried, Germany

Glycosylation is a universal strategy to post-translationally modify proteins. We discovered a new form of N-linked glycosylation that activates the polyproline specific bacterial translation elongation factor EF-P. The modifying enzyme was identified as the EF-P arginine rhamnosyltransferase EarP. The reaction catalyzed by EarP represents several novel concepts in protein N-glycosylation. Classically, this form of post-translational modification occurs on asparagine moieties of extracellular and membrane proteins. The target sites for the universal glycosyltransferase are determined by a highly conserved sequon with the primary structure N-X-S/T. Conversely, EF-P - the acceptor substrate of EarP - is the first bacterial protein that is modified at an arginine and the first example of an N-glycosylated cytosolic protein. It also lacks a comparable target sequon indicating that the specialized rhamnosyltransferase EarP might utilize an alternative mechanism of acceptor substrate recognition. We previously showed that the KOW-like EF-P N-domain is sufficient for recognition and modification. Here, we generated successive truncations of the *Pseudomonas putida* EF-P acceptor loop and identified an eleven amino acids long minimal target peptide consisting of the seven amino acid acceptor loop and two amino acids of both the preceding and following beta strand. Using small peptides, we confirmed this minimal motif and investigated both the variability of the sequence context and the applicability of these variants as specific inhibitors of the rhamnosyl transfer reaction. Apart from this, our structural and biochemical analysis of EarP suggests that the glycosyltransferase exhibits natural substrate promiscuity towards the nucleotide sugar, possibly accepting not only TDP-β-L-rhamnose but also alternative donors. Together, these findings lay the basis for engineering EarP into a glycosynthase for targeted glycosylation of various proteins.

---

### 346-OTP

**Microbial Sulfur Oxygenase Reductases: Structures, Identification of Reaction Intermediates and Implications for the Reaction Mechanism**

\*A. Kletzin<sup>1</sup>, P. Rühl<sup>1</sup>, K. Parey<sup>2,3</sup>, F. Folgosa<sup>4</sup>, M. Teixeira<sup>4</sup>, J. Vonck<sup>3</sup>, C. Frazão<sup>5</sup>

<sup>1</sup>Technische Universität Darmstadt, Mikrobiologie, Darmstadt, Germany

<sup>2</sup>Goethe University Medical School, Structural Bioenergetics Group, Frankfurt am Main, Germany

<sup>3</sup>Max-Planck-Institute of Biophysics, Dept. of Structural Biology, Frankfurt am Main, Germany

<sup>4</sup>ITQB NOVA, Metalloproteins and Bioenergetics Unit, Oeiras, Portugal

<sup>5</sup>ITQB NOVA, Structural Biology Laboratory, Oeiras, Portugal



**Introduction:** Soluble sulfur oxygenase reductases (SOR) are the initial enzymes in sulfur oxidation pathways of the thermoacidophilic archaeon *Acidianus ambivalens* (AaSOR) and of the mesophilic Gammaproteobacteria *Halothiobacillus neapolitanus* (HnSOR) and *Thioalkalivibrio paradoxus* (TpSOR); the latter being an alkaliphile. They catalyze the oxygen-dependent disproportionation of elemental sulfur and polysulfide with sulfite, thiosulfate and hydrogen sulfide as products at optimal temperatures of around 80°C and at stoichiometries of 4-10:1 (oxidized:reduced products).

**Methods:** 3D structures were determined of TpSOR and AaSOR (cysteine-derivatized with inhibitors) by X-ray crystallography and cryo-electron microscopy. *In silico* docking of was performed with polysulfide.

**Results:** The TpSOR, which branches most deeply in dendrograms of the protein family, is the only SOR with little reductase activity ( $\geq 30:1$ ) but it has the highest oxygenase activity of all SORs so far ( $\geq 300$  U/mg protein vs.  $\approx 80$  U/mg for HnSOR and  $\approx 40$  U/mg for AaSOR). All SORs are globular ball-shaped 24-subunit thermozyms with melting points of  $\approx 80^\circ\text{C}$  for Tp- and HnSOR and  $\geq 100^\circ\text{C}$  for the AaSOR.

The active sites in each subunit of the SORs comprise a mononuclear low-potential non-heme iron (midpoint potentials of  $-252 - -265$  mV) and three conserved cysteines, which are localized in a spacious pocket in the Aa- and HnSORs, whereas this is more constricted and shaped differently in the TpSOR. Cysteine persulfide modifications were not identified in the TpSOR. Structure comparison showed an overall similar configuration of TpSOR to Aa- and HnSORs but with many minor differences, e.g. in the channel networks within the proteins and the orientation of the cysteines. Polysulfide docking suggested substrate binding and import into the active site pocket via the essential cysteine residue C31/33/44 (Aa-, Hn-, TpSOR).

**Conclusions:** The other two conserved cysteines C101 and C104 (AaSOR) are suggested to participate in sulfane sulfur (de-)polymerization reactions. Further conclusions concerning the reaction mechanism, the thermostabilities and the differences between the SORs will be discussed.

### 347-OTP

#### A network of sulfurtransferases delivering sulfur to the sulfur-oxidizing heterodisulfide reductase-like (sHdr) complex

\*W. Falßbender<sup>1</sup>, E. Bach<sup>1</sup>, S. Tanabe<sup>1</sup>, M. Grosser<sup>1</sup>, C. Dahl<sup>1</sup>, L. Hahn<sup>1</sup>

<sup>1</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Mikrobiologie & Biotechnologie, Bonn, Germany

A heterodisulfide reductase-like complex and novel lipoate-binding proteins (LbpAs) are central players of a wide-spread pathway of dissimilatory sulfur oxidation.<sup>1,2</sup> It occurs in a huge organism group that includes the volatile organic sulfur compound degrader *Hyphomicrobium denitrificans* and many chemo- and photolithoautotrophic bacteria and archaea. The *shdr-lbpA* genes are always associated with genes for accessory sulfurtransferases (DsrE proteins, TusA, rhodanases). Related proteins have an established role in the Dsr-pathway of sulfur oxidation, where they act together in transferring sulfur as a cysteine persulfide onto DsrC that finally presents the sulfur to the oxidizing enzymatic unit, DsrAB.<sup>3</sup> Here, we asked whether sulfur delivery to the sHdr-complex proceeds in a similar manner. A toolbox of sHdr-linked recombinant DsrE, TusA and LbpA proteins from *H. denitrificans* and the lithoautotrophs *Thioalkalivibrio* sp. K90mix and *Thiorhodospira sibirica* was created. The

homotrimeric DsrE3B proteins from *Thioalkalivibrio* and *T. sibirica* donated sulfur to TusA as shown by mass spectrometry. Other sulfur transfer reactions also occurred *in vitro*, with TsDsrE3B acting as an especially efficient donor to the lipoylated holo-LbpA2 protein from the same organism. The *in vivo* role of sulfur transfer reactions was studied in the genetically accessible *H. denitrificans*. LbpA had already been proven essential for sulfur oxidation.<sup>2</sup> Here, we replaced the gene by variants with cysteine to alanine exchanges at two conserved positions. Both cysteines proved indispensable for sulfur oxidation. Removal of the *hdr*-associated *dsrE* gene also led to a sulfur-oxidation negative phenotype. The *tusA* gene could not be deleted. *H. denitrificans* has only a single copy of this gene and the protein probably serves multiple physiological functions necessary for viability just as has been observed for *Allochromatium vinosum*.<sup>3</sup> In summary, our results provide first insights into an extensive network of sulfur transfer reactions involved in delivery of sulfur to the oxidizing sHdr system.

<sup>1</sup>Koch, Dahl 2018 ISME J 12, 2479

<sup>2</sup>Cao et al 2018 eLife 7, e37439

<sup>3</sup>Stockdreher et al 2014 JBC 289, 12390

### 348-OTP

#### DNAzymes as catalysts for L-tyrosine and amyloid beta oxidation

\*T. Köhler<sup>1</sup>, J. Thiele<sup>2</sup>

<sup>1</sup>Leibniz-Institut für Polymerforschung, Nanostrukturierte Materialien, Dresden, Germany

<sup>2</sup>Leibniz Institute of Polymer Research, Nanostructured Materials, Dresden, Germany

### Question

Here, we present an oxidation reaction for DNAzymes that are single-stranded DNA (ssDNA) having a catalytic activity when hemin is incorporated. We investigated the oxidation of L-tyrosine to dityrosine by hydrogenperoxide (H<sub>2</sub>O<sub>2</sub>) which can be catalyzed by DNAzymes. We screened for optimal stoichiometry of hemin in relation to ssDNA and investigated different sequences for their catalytic activity. These optimized parameters were used to apply this model reaction to the oxidation of amyloid beta (A $\beta$ ). That way, we can investigate a possible effect of DNAzymes in neurodegenerative diseases.

### Methods

L-tyrosine oxidation was detected by fluorescence increase at 405 nm by using a plate reader. After optimizing the oxidation of L-tyrosine and its detection, we investigated the oxidation of A $\beta$  by the same measurement setup. Furthermore, we investigated the oxidation of L-tyrosine in the presence of fetal calf serum to underline its physiological meaningfulness

### Results

We found out that the fluorescence spectra of free L-tyrosine as well as the tyrosine within the peptide sequence of A $\beta$  showed significant changes, when oxidized by free hemin as well as hemin being incorporated in DNAzymes. We observed an optimal stoichiometry of hemin-to-ssDNA of 1:10 to form a catalytically active DNAzyme. Further, we did not find an influence when adding only a single base to the

DNAzyme sequence. Additionally, we could show that A $\beta$  can be oxidized by hemin as well as synthetic or natural DNAzyme sequences.

## Conclusions

We present that DNAzymes acting as suitable catalysts for the synthesis of dihydroxyphenylserine by oxidizing L-tyrosine with H<sub>2</sub>O<sub>2</sub>. We further suggest a possible influence of DNAzymes in patho- and physiological processes that was exemplarily shown by the oxidation of A $\beta$  and oxidation of L-tyrosine in the presence of fetal calf serum.

---

### 349-OTP

#### Biosecurity Implications of Genome Editing Technologies

\*M. Himmel<sup>1</sup>

<sup>1</sup>University of Hamburg, Centre for Science and Peace Research & Dept. for Microbiology and Biotechnology, Hamburg, Germany

New genome editing tools such as CRISPR/Cas are thought to allow genome manipulation with previously unthinkable precision and efficiency. Genome editing is therefore categorized as emerging technology due to its promising features, broad range of possible application and level of maturation [1]. On the other side, the US Intelligence Community stated in the 2016 Worldwide Threat Assessment that genome editing "probably increases the risk of the creation of potentially harmful biological agents and products" [2]. The scientific community is heavily engaged in promoting the peaceful use of this technology and supports the mitigating of misuse of genetic engineering for hostile purposes. For better understanding of risks and benefits associated with genome editing, a thorough risk assessment is required. Here, we address the question how to assess potential biosecurity threats emanating from genome editing techniques in a systematic approach including lab-based studies. The results obtained will support the informed scientific and societal debate about genome editing technologies.

[1] Himmel, M. (2019). Emerging Dual-Use Technologies in the Life Sciences: Challenges and Policy Recommendations on Export Control. Non-Proliferation and Disarmament Papers, (64).

[2] Clapper, J. R. (2016). Statement for the Record on the Worldwide Threat Assessment of the US Intelligence Community for the House Permanent - Select Committee on Intelligence. Office of The Director of National Intelligence Washington DC.

---

### 350-OTP

#### Microbiology literacy: Conceptions of high school students about bacteria

C. Höllmann<sup>1</sup>, S. Kühn<sup>1</sup>, W. Manz<sup>1</sup>, \*J. Meier<sup>1</sup>, M. Mikloweit<sup>1</sup>, C. Thiel<sup>1</sup>

<sup>1</sup>University Koblenz-Landau, Institute for Integrated Natural Sciences, Koblenz, Germany

In contrast to the significance of microbiology for global life, science and university education, microbiology often plays only a diminutive role when it comes to high school education. In the German federal state of Rhineland-Palatinate, the biology syllabus only briefly touches single aspects of microorganisms which are embedded into very different contexts (e.g. cell structure, infection and immune system). Microbiology as genuine discipline as well as

important issues of microbiologically mediated ecosystem functions and services are completely neglected. This is diametrically opposed to the societal challenges we are facing today including climate change, spread of antibiotic resistance and emerging pollutants which are urgently demanding for sufficient microbiology literacy [1]. Against this background we conducted a comprehensive survey among 660 high school students visiting grade 9 and 10 in order to investigate their conceptions of bacteria. In the questionnaire the students were requested to create a mind map and to sketch a bacterium. Furthermore, they were asked to comment on size and occurrence, the different features of life, and the relationship between bacteria and e.g. antibiotics, fever, and clean water. The preliminary data analysis indicated that the majority of the students have a conception of bacteria as living organisms. From the students' perspective the bacteria appear to be ubiquitously present and to proliferate. However, interactions with the environment were often denied. Most students considered bacteria as both "good" and "bad". While the statements were quite precise reflecting the disadvantages of bacteria (causing diseases), the answers explaining the necessity of bacteria for our well-being remained vague. Only scarce knowledge was present about the role of bacteria in the environment. The presentation will be highlighting main characteristics of the students' conceptions about bacteria, analyzing these in respect to their compliance with scientific concepts, and identifying misconceptions and their possible sources (school-based or extracurricular). [1] Timmis et al. (2019) Environ Microbiol 21:1513-1528

---

### 351-OTP

#### The verification of scientific data – does it make sense?

\*B. J. Tindall<sup>1</sup>

<sup>1</sup>Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH., Braunschweig, Germany

An ever increasing flood (Tsunami is a better description) of data it is making it increasingly difficult for individual scientist to maintain the overview. The title highlights two aspects, one whether it makes sense to verify data and secondly whether the data one has obtained make sense. It should be self-explanatory that it makes sense to verify data, but there is evidence that this is not always the case and often comes to light when data deposited in databases or in publications contain data that appears to be "anomalous". Those who detect "anomalous" data are in fact trying to verify previous deposited/published work and recognising the fact that a data set is anomalous also indicates that the second question has been asked - whether the original data makes sense in the context in which it was deposited/published. Failing to either realise that a data set has not been verified or that the data does not fit in the biological context claimed (ie is "anomalous") can often affect the way data is interpreted and theories and hypotheses developed. Examples will be given illustrating the importance of putting data in an evolutionary and biological context rather than accepting data deposited in a database or in a publication at face value.

---

### 352-OTP

#### AmpGram: a novel tool for prediction of antimicrobial peptides

M. Burdukiewicz<sup>1</sup>, \*J. Chilimoniuk<sup>2</sup>, K. Sidorczuk<sup>2</sup>, F. Pietluch<sup>2</sup>, D. Rafacz<sup>1</sup>, S. Rödiger<sup>3</sup>, P. Gagat<sup>2</sup>

<sup>1</sup>Warsaw University of Technology, Warsaw, Poland

<sup>2</sup>University of Wrocław, Wrocław, Poland

<sup>3</sup>Brandenburg University of Technology Cottbus - Senftenberg, Senftenberg, Germany

**Question:** Antimicrobial peptides (AMPs) are ancient and evolutionarily conserved molecules widespread in all living organisms that participate in host defense and/or microbial competition. Due to their positive charge, hydrophobicity and amphipathicity, they preferentially disrupt negatively-charged bacterial membranes. AMPs are considered an important alternative to traditional antibiotics, especially in times when the latter is drastically losing their effectiveness. Therefore, efficient computational tools for AMP prediction are essential to identify the best AMP candidates without undertaking expensive experimental studies.

**Methods:** AmpGram is our novel tool for predicting AMPs based on the methodology that has already been used with success in our previous applications. It employs simplified alphabets to encode the information from highly variable AMPs into informative features suitable for machine learning. Next, n-gram analysis reveals amino acid motifs associated with the presence or absence of antimicrobial properties.

**Results:** In order to test AmpGram performance, we benchmarked it against the state-of-the-art AMP classifiers, including AMPScanner 2.0, CAMPR3(RF) and iAMPpred. AmpGram outperformed all of them with AUC 0.94. Additionally, AmpGram provides a list of amino acid motifs associated with antimicrobial properties.

**Conclusions:** AmpGram is a novel tool for the prediction of antimicrobial peptides outperforming existing tools. Moreover, it identifies amino acid motifs that can be used to study existing AMPs or to design new ones.

**Acknowledgments:** This project was funded by National Science Center (2017/24/T/NZ2/00003).

---

### 353-GIP

#### **Gut Inflammation is Causing a Change in the Microbiome of *Manduca sexta***

\*A. G. Müller<sup>1</sup>, T. Trenczek<sup>1</sup>

<sup>1</sup>Justus Liebig University of Giessen, General Zoology and Developmental Biology, Giessen, Germany

#### **Question**

A disturbance of the gut microbiome regularly accompanies inflammatory bowel diseases. However, it is still in debate whether this bacterial disturbance is the cause or a consequence of inflammatory bowel diseases. The purpose of the present study is to use the insect *Manduca sexta* as a model for inflammatory bowel diseases and to examine if an experimental inflammatory bowel like gut inflammation is leading to a change in the gut microbiome of *M. sexta*.

#### **Methods**

We have previously shown that the oral application of uracil is inducing an inflammatory bowel like gut inflammation through the production of ROS in *M. sexta*. Bacteria from feces of *M. sexta* fed with uracil (n = 12) and control diet (n = 14) were isolated and characterized via 16S rRNA Gene sequencing. CFUs of the feces from experimental and control larvae were quantified and analyzed.

#### **Results**

The bacterial community of *M. sexta* feces has not been characterized till now. The community was essentially composed of Actinomycetales and Lactobacillales.

Rarefaction curve of ACE and Chao 1 showed saturation. There was a significant reduction of the bacterial load (p = 0.0037), as well as a perturbation within the *Enterococcus* community (p = 0.0211) in uracil treated animals.

#### **Conclusions**

We show that an inflammatory bowel like gut inflammation in *M. sexta* is leading to a total reduction and a community shift in gut bacteria. These results indicate that inflammation on its own can change the abundance and composition of colon bacteria and may lead to a better understanding of inflammatory bowel diseases.

---

### 354-GIP

#### ***Escherichia coli* Nissle 1917 encodes a type VI secretion system which is active in vitro and might help fight gastrointestinal pathogens.**

\*T. Oelschlaeger<sup>1</sup>, C. Niklas<sup>2</sup>

<sup>1</sup>University of Würzburg, Institut für Molekulare Infektionsbiologie, Würzburg, Germany

<sup>2</sup>University of Würzburg, Zentrum für Infektionsforschung, Würzburg, Germany

*Escherichia coli* Nissle 1917 encodes a type VI secretion system which is active in vitro and might help fight gastrointestinal pathogens.

#### **Introduction**

The probiotic *E. coli* strain Nissle 1917 (EcN) is licensed as a medication for the treatment of gastrointestinal diseases e.g. colitis ulcerosa and diarrhea. However, many of its properties involved in its probiotic nature have to be identified. One class of factors which might be responsible for EcN's ability to kill pathogenic bacteria is peptides and proteins secreted by EcN and toxic for other bacteria such as the microcins H47 and M.

#### **Objectives**

Based on EcN's genome sequence we identified a gene cluster encoding a type VI secretion system (T6SS) which was shown for other *E. coli* to kill prey bacteria in a contact dependent manner. We wondered if EcN's T6SS is active in vitro resulting in killing of prey bacteria.

#### **Material & Methods**

Equal numbers of killer bacteria (SK22D, the microcin-negative mutant of EcN, UPEC strain CFT073 the closest relative of EcN, *Pseudomonas aeruginosa*) or the T6SS-negative control strain MG1655 and prey (*E. coli* DH5α) bacteria were mixed and 20 µl of the mixture were spotted on an agar plate. After 5 h at 37°C the mixture was transferred from the plate into 1 ml LB medium and serial dilutions were plated on agar plates containing an antibiotic allowing only growth of the prey. The resulting colonies were used to calculate the percentage of surviving prey bacteria.

#### **Results**

The cfu of DH5α after coinoculation with MG1655 was defined as 100 %. As expected *P. aeruginosa* killed the prey bacteria (70 % up to 87 % killed). More efficient was CFT073 (> 99% killing). SK22D killed between 92 % and 99 % of the prey bacteria.

## Conclusion

Even the microcin-negative mutant of EcN (SK22D) kills prey bacteria more efficient than *P. aeruginosa* but less efficient than CFT073. This effect is most likely due to the presence of the T6SS gene cluster of EcN. The construction of a T6SS-negative mutant is under construction to prove the observed killing to be based on the expression of the T6SS.

---

## 355-GIP

The author has not agreed to a publication.

---

## 356-GIP

The role of the accessory gene *cagP* for the *Helicobacter pylori* Cag type IV secretion system

\*V. Beißwenger<sup>1</sup>, E. Weiss<sup>1</sup>, W. Fischer<sup>1</sup>

<sup>1</sup>Max von Pettenkofer Institute, Chair of Medical Microbiology and Hospital Epidemiology, LMU, Munich, Germany

Most strains of the human gastric pathogen *Helicobacter pylori*, which infects approximately 50% of the world population, harbour the cytotoxin-associated gene (*cag*) pathogenicity island, a major virulence determinant and risk factor for development of gastric adenocarcinoma. The *cag* pathogenicity island encodes 20 components of a type IV secretion system, as well as the translocated effector protein CagA. Apart from these essential proteins, several accessory genes have been found to support type IV secretion-associated functions to different degrees, although their functional role has not been elucidated so far. In this study, we have analyzed one of these genes, *cagP*, which is required for full function of the secretion apparatus in *H. pylori*.

We have shown this via a TEM-1- $\beta$ -lactamase reporter assay, which was used to determine the CagA translocation in different mutants of strain P12, that show a complete or partly lack of the *cagP* gene locus. The assay was also used to explore the role of a small non-coding RNA encoded upstream of *cagP*.

While a deletion of *cagP* prevents formation of a fully functional Cag type IV secretion system, this is not the case for a deletion of the small RNA. Deletion of *cagP* also decreases the expression of *cagH*, *cagI* and *cagL*, putative assembly factors for the pilus structures. The influence of *cagP* on CagH was shown using a NanoLuc luciferase reporter system (HiBiT assay).

To obtain evidence about the expression of *cagP* and its regulation the HiBiT assay was also used to evaluate the protein concentrations of CagP. HiBiT reporter strains were incubated either with or without host cells and luminescence was measured afterwards. Upon infection of AGS cells CagP levels were increased. Those experiments were also performed with *cagG*, another accessory gene encoded in the pathogenicity island, which showed an increase as well.

Taken together, our data reveals the influence the gene locus of *cagP* has on the Cag type IV secretion system activity. Furthermore it could be established, that *cagP* expression can be modulated by environmental conditions, which might be important for the regulation of the whole secretion system.

## 357-GIP

Exploring the structural diversity of mucin-derived O-glycans by reductive elimination and mass spectrometric analysis

\*G. Pohlentz<sup>1</sup>, M. Mormann<sup>1</sup>, S. Kruse<sup>1</sup>, J. Mühling<sup>1</sup>

<sup>1</sup>Institute for Hygiene, Institute of Hygiene, Glycobiology Group, Münster, Germany

**Introduction:** Mucus covers the epithelia of, e.g., the respiratory and the gastrointestinal tract. Its main constituents are mucins, a class of heavily O-glycosylated proteins. The dense glycan coat of mucins protects the underlying epithelium from pathogenic and biochemical attack or chemical damage. Moreover, especially the O-glycans are supposed to serve as attachment sites and as nutrients for commensal and pathogenic bacteria. Investigations on the bacteria-glycan interplay require the in depth analysis of the O-glycan structures.

**Objective:** In the present study we performed a combination of reductive elimination and mass spectrometry to get hands on the O-glycans of mucins.

**Materials and Methods:** O-glycans from porcine stomach and bovine submaxillary gland, respectively, were liberated from the protein core by reductive elimination using NaBH<sub>4</sub>. After removal of boric acid glycans were purified by solid phase extraction on i-HILIC. Subsequent nano electrospray ionization mass spectrometry (ESI MS) was performed with a Synapt GS-2 instrument.

**Results:** More than 40 different O-glycans derived from porcine and bovine mucin, respectively, were identified, not including isomeric and isobaric structures. The detected glycans ranged from low-end di- and trisaccharides of different core types to complex oligosaccharides composed of 14 monosaccharides. Porcine glycans contained solely neutral sugars (Gal, GalNAc, GlcNAc, Fuc), while bovine glycans exhibited a number of sialylated species additionally comprising N-acetylneuraminic acid (Neu5Ac) or N-glycolylneuraminic acid (Neu5Gc).

**Conclusion:** The results of the present study demonstrate that the combination of reductive elimination and state-of-the-art mass spectrometry provides an excellent tool for structure elucidation of mucin-derived O-glycans.

---

## 358-GIP

Bile acid stress response of *Clostridioides difficile* and its effects on host-pathogen interaction

N. Metzendorf<sup>1</sup>, S. Dittmann<sup>1</sup>, R. Schlüter<sup>2</sup>, K. Riedel<sup>1</sup>, \*S. Sievers<sup>1</sup>

<sup>1</sup>University of Greifswald, Institute of Microbiology, Microbial Physiology and Molecular Biology, Greifswald, Germany

<sup>2</sup>University of Greifswald, Imaging Center, Greifswald, Germany

**Introduction:** *Clostridioides difficile* is an intestinal human pathogen that infects the gut of individuals with a depleted microbiota. Bile acids of the intestinal tract have been demonstrated to play an important role in the initiation and outcome of a *C. difficile* infection.

**Objectives:** Characterization of the specific response of vegetative *C. difficile* cells to long-term (LT) bile acid stress and the effect on host-pathogen interactions.

**Materials & methods:** Proteome signatures of *C. difficile* cells exposed to long-term (LT) stress were obtained by LC-MS/MS. The four main bile acids cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA) and lithocholic acid (LCA) were tested. Bacterial morphology was

determined and quantified in electron micrographs (EM). Co-cultivation experiments of bile acid stressed *C. difficile* with intestinal endothelial cells (CaCo-2 cells) were performed and analyzed by EM and fluorescence microscopy.

**Results:** LT stress caused a significant and bile acid specific change in global protein signatures. The major structural flagellum protein FliC was dramatically decreased in the presence of LCA compared to control conditions, while DCA and CDCA resulted in moderate down-regulation of FliC, and CA did not affect FliC expression at all. EM validated these findings: while CA treatment did not alter the number of flagella, DCA- and CDCA-stressed *C. difficile* had fewer, and LCA-treated bacteria were almost devoid of flagella compared to the control. Co-cultivation experiments showed that bacteria with flagella were in direct interaction with the host.

**Conclusion:** Being involved in motility, adherence and immunogenicity, flagella represent a major virulence determinant of pathogenic bacteria. We found a differential impact of bile acids on the formation of flagella in *C. difficile*. The individual bile acid composition of people could therefore have a large impact on the virulence of this pathogen.

### 359-GIP

#### Autopenetration of *Shigella* effector protein IpaH7.8 and modulation of signaling responses in cellular barriers.

\*N. Y. Ivan<sup>1</sup>, N. Stefanie<sup>1</sup>, M. Theresa<sup>1</sup>, R. Christian<sup>1</sup>

<sup>1</sup>Institute for Infectiology, Zentrum für Molekularbiologie der Entzündung (ZMBE), Münster, Germany

#### Introduction:

Effector proteins are key virulence factors of pathogenic bacteria that target and subvert the functions of essential host defense mechanisms after breaking cellular barriers. Typically, these proteins are delivered into infected host cells via the type III secretion system (T3SS). Recently, however, several effector proteins have been found to enter host cells in a T3SS-independent manner thereby widening the potential functional range of these virulence factors. Prototypes of such bacteria-derived cell-penetrating effectors (CPEs) are the *Y. enterocolitica*-derived YopM as well as the *S. typhimurium* effector SspH1.

#### Objectives:

Both YopM and SspH1 belong to bacterial effectors of the LPX subtype of leucine-rich repeat (LRR) proteins which also comprises different IpaH proteins of *Shigella*. Due to significant homology in sequence and structure, we suggested a general concept for T3SS-independent uptake of LPX effector proteins and confirmed in a recent study their ability to autonomously translocate into eukaryotic cells.

#### M&M:

Here, we would like to provide further evidence of a general concept of T3SS-independent translocation of bacterial LPX effector proteins, with particular emphasis on *Shigella* IpaH7.8 effector protein. For this purpose, we generated several truncated versions of IpaH7.8. The purified proteins were used to assess their ability to cross the host membrane by FACS and fluorescence microscopy. By ubiquitination proteomics, we analyzed their enzymatic functionality as well as their modulation of host cell signaling pathways.

#### R&C:

We confirmed the ability of IpaH7.8 to autonomously translocate into eukaryotic cells. Moreover, our results point to a major contribution of endocytosis to T3SS-independent cellular uptake of IpaH7.8. The N-terminal  $\alpha$ -helices of IpaH7.8 were identified as a protein transduction domain (PTD). Upon cell-penetration, IpaH7.8 is enzymatically functional and poly-ubiquitinates Glomulin, a negative regulator of the inflammasome. Taken together, along with their natural capacity to modulate host immune signaling pathways, *Shigella* effector proteins might serve as potential "self-delivering" biological therapeutics.

### 360-GIP

Potential of cell-free supernatants from gut commensal bacteria as inhibitor of *C. difficile* toxin production

\*A. Breitrück<sup>1,2</sup>, K. Krüger<sup>2</sup>, B. Kreikemeyer<sup>1</sup>

<sup>1</sup>University Medicine Rostock, Institute of Medical Microbiology, Virology and Hygiene, Rostock, Germany

<sup>2</sup>Fraunhofer Institute for Cell Therapy and Immunology IZI, Extracorporeal Immunomodulation, Rostock, Germany

#### Introduction

*Clostridioides difficile* (*C. difficile*) is the most frequent cause of nosocomial antibiotic-associated diarrhea. The incidence of *C. difficile* infection (CDI) has been rising worldwide with subsequent increases in morbidity, mortality and health care costs. Depleting gut microbiota by antibiotic therapy, favors *C. difficile* germination, colonization and consecutive toxin production leading to clinical symptoms of CDI.

#### Objectives

In this study we investigate the direct effect, as well as cell-free supernatant (CFS) of three commensal bacteria from Firmicutes (*Faecalibacterium prausnitzii*, *Roseburia intestinalis*) and Bacteroidetes (*Bacteroides faecis*) phylum on *C. difficile* growth and toxin production. Furthermore, the vitality of human intestinal epithelial cells under *C. difficile* toxins exposition w/o supernatant from commensals was assessed.

#### Material

Three commensal bacteria species and two strains of *C. difficile* were grown under anaerobic conditions. The living commensal bacteria as well as their CFS derived from cultures, were tested for inhibition of *C. difficile* by incorporating various proportions of the CFS into the growth medium with co-cultures of the pathogen. The effect on growth and toxin production were assessed. The biomass of CaCo-2 cells was determined after co-incubation of cells with *C. difficile* toxin containing media w/o CFS.

#### Results

Experimental results indicated that in all co-cultures the toxin production was decreased, while no decrease in the number of colonies could be observed. The same effects could be observed in a concentration-dependent manner by using CFS from the commensal strains only. Furthermore, the cytotoxic effect of *C. difficile* toxins on CaCo-2 could be reduced by co-incubation with CFS.

#### Conclusion

All three species of commensal bacteria investigated in this study showed potential for targeting the toxin-production of

*C. difficile*. CFS derived from these commensals was responsible for the inhibition of toxin production. Further studies will help to identify specific substances from the CFS as potential candidates in the search for alternatives to conventional CDI-therapy with antibiotics.

### 361-GIP

#### Development of cell-permeable antimicrobial molecules (CPAMs) to treat intracellular infections

\*C. Rüter<sup>1</sup>, T. Martins<sup>1</sup>, B. Körner<sup>2</sup>, L. Greune<sup>2</sup>, A. Schmidt<sup>1</sup>

<sup>1</sup>ZMBE, Institute of Infectiology, Münster, Germany

<sup>2</sup>Universitätsklinikum Münster, Institut für Infektiologie, ZMBE, Münster, Germany

Infectious diseases caused by microbial pathogens, are one of the major causes of morbidity and mortality worldwide. However, they represent a great challenge for antimicrobial therapies not only due to the threat of spreading antimicrobial resistances but also due to the fact that some major bacterial pathogens can also adopt an intracellular lifestyle. Thus, intracellular pathogens are widely protected against the usual antimicrobial therapeutics and they constitute a niche for recurrence and reinfection. Commonly used antimicrobial agents are characterized by poor cellular uptake. They cannot efficiently reach the intracellular target, resulting in low antimicrobial activity against intracellular pathogens. Therefore, there is a need to develop and improve strategies for the treatment of such infectious diseases. In this regard, Cell-penetrating peptides (CPPs), also called protein transduction domains (PTDs), are small peptides that are able to cross the plasma membrane autonomously and can mediate the internalization of biologically active molecules such as antibiotics into the cytoplasm.

In our previous work, we discovered novel bacteria-derived PTDs within the N-terminal  $\alpha$ -helical domains of LPX-effector proteins and used these PTDs for the delivery of the commonly used antibiotic gentamicin to treat intracellular infections with bacterial pathogens. We showed that these CPP-gentamicin conjugates were able to penetrate different endothelial and epithelial cell lines and importantly, we confirmed that these conjugates were able to target and efficiently kill intracellular Gram-negative pathogenic bacteria, such as *E. coli* K1 RS218, *S. enterica* serovar Typhimurium, and *S. flexneri* in infected cells. Furthermore, we could show that bacteria-derived peptidoglycan-degrading proteins (PGDs) fused to a CPP can be used to kill intracellular pathogenic Gram pos. and Gram neg. bacteria, as well. Interestingly, these fusion constructs also showed a high effectivity against bacterial biofilm formation and might represent novel therapeutic tools to treat (recurrent) infectious diseases caused by bacteria.

### 362-RCP

#### Challenges in the identification of a novel *Leclercia* species are only resolved using a hybrid whole-genome sequencing approach

\*A. Viehweger<sup>1</sup>, M. Hönemann<sup>2</sup>, N. Dietze<sup>1</sup>, J. Johnke<sup>3</sup>, B. König<sup>1</sup>, A. C. Rodloff<sup>1</sup>

<sup>1</sup>Leipzig University Hospital, Medical Microbiology and Epidemiology of Infectious Diseases, Leipzig, Germany

<sup>2</sup>Leipzig University, Virology, Leipzig, Germany

<sup>3</sup>Christian Albrechts University Kiel, Evolutionary Ecology and Genetics, Kiel, Germany

Introduction: Strain 4-9-1-25 was isolated from an infant with a severe presentation of pneumonia and septicemia at the Leipzig University Hospital. The strain was initially identified

by MALDI-TOF analysis as *Leclercia adecarboxylata*, the sole member of the genus *Leclercia*. However, analysis of the 16S rRNA via Sanger sequencing yielded inconclusive results.

Objectives: We, therefore, performed hybrid whole-genome sequencing to characterize the isolate, which illustrated general caveats of 16S rRNA analysis.

Methods: We amplified a 16S rRNA fragment of 1,400 bp for phylogenetic characterization of strain 4-9-1-25 and five comparative isolates, complemented by identification based on substrate usage. The genome of strain 4-9-1-25 was reconstructed using hybrid Illumina and Nanopore sequencing. Taxonomy was resolved using a k-mer based search algorithm against an extensive genome collection and validated using marker genes and *in silico* DNA-DNA hybridization.

Results: All analyzed strains did not differ biochemically. The analysis of the partial 16S rRNA gene, however, was inconclusive for strain 4-9-1-25 due to assignment to different species of the Enterobacterales. Additionally, multiple ambiguous nucleotides were detected for all isolates due to intragenomic heterogeneity of 16S rRNA genes, which was only revealed using haplotype reconstruction with Nanopore long reads. The final assembly of the circular genome contains 4.4 Mbp and has an average nucleotide identity below 95% to any known isolate in a comprehensive genome collection of Enterobacterales.

Conclusion: Low resolution and intra-genomic heterogeneity can limit discrimination of bacterial isolates when using only 16S rRNA genes. We successfully applied a hybrid sequencing approach to mitigate these problems. Based on this whole-genome analysis, we propose that strain 4-9-1-25 represents a new species of the genus *Leclercia*.

### 363-RCP

Polyphasic taxonomy of an antibiotic-producing

#### *Streptomyces* sp. SHP 1-2

\*C. Risdian<sup>1,2</sup>, T. Mozeš<sup>3</sup>, S. Ratnakomala<sup>4</sup>, P. Lisdiyanti<sup>4</sup>, R. L. Hahnke<sup>5</sup>, M. Rohde<sup>6</sup>, J. Wink<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Infection Research, Microbial Strain Collection, Braunschweig, Germany

<sup>2</sup>Indonesian Institute of Sciences (LIPI), Research Unit for Clean Technology, Bandung, Indonesia

<sup>3</sup>Indonesian Institute of Sciences (LIPI), Research Center for Chemistry, Serpong, Indonesia

<sup>4</sup>Indonesian Institute of Sciences (LIPI), Research Center for Biotechnology, Bogor, Indonesia

<sup>5</sup>Leibniz Institut DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany

<sup>6</sup>Helmholtz Centre for Infection Research, Central Facility for Microscopy, Braunschweig, Germany

*Streptomyces* belong to phylum Actinobacteria and are also known as important genera in medicine because of their ability to produce more than two-thirds of the antibiotics in the world. The chance for finding novel bioactive compounds produced from new filamentous Actinobacteria is relatively high when the discovery approaches are aimed to neglected and unexplored regions. Strain SHP 1-2 was isolated from Enggano Island, Indonesia, and had antimicrobial activity. The strain was found to be closely related to the type strains of *Streptomyces viridochromogenes* NBRC 3113(T) (99.03%), *Streptomyces malachitofuscus* NBRC 13059(T) (99.03%), and *Streptomyces misionensis* DSM 40306 (T) (98.96%). The cell wall contained LL- diaminopimelic acid. The major fatty acids were anteiso-C15:0, iso-C16:0, anteiso-C17:0, iso-C15:0, and C16:0. The primary

menaquinones were MK-9(H4), MK-9(H6), and MK-9(H8). The G+C content was found to be 73.37%. The multilocus sequence analysis (MLSA) distance using five house-keeping genes of strain SHP 1-2 and the closest strains were less than 0.007. After studying polyphasic taxonomy, that includes the genotypic and phenotypic comparison between strain SHP 1-2 and the closest strains, it can be concluded that the strain SHP 1-2 is a novel species in the *Streptomyces* genus.

**Keywords:** *Streptomyces*, Actinobacteria, polyphasic taxonomy, Enggano Island, and antimicrobial activity.

---

### 364-RCP

#### The EDGAR platform for phylogenomics

\*J. Blom<sup>1</sup>, M. Dieckmann<sup>1</sup>, J. G. Schrader<sup>1</sup>, S. P. Glaeser<sup>2</sup>, A. Goesmann<sup>1</sup>

<sup>1</sup>Justus-Liebig-University Gießen, Bioinformatics & Systems Biology, Gießen, Germany

<sup>2</sup>Justus-Liebig-University Gießen, Microbiology of Recycling Processes, Gießen, Germany

The deployment of next generation sequencing approaches has caused a rapid increase in the number of completely sequenced genomes. As one result of this development, it is feasible to analyze not only single genomes, but large groups of related genomes in a comparative approach. Whole genome sequencing of type strain genomes also holds huge potential for obtaining a higher resolution phylogenetic and taxonomic classification. In the last 10 years, the EDGAR platform has become one of the most established software tools in the field of comparative genomics. Since, the software has been continuously improved and a large number of new analysis features have been added. In recent years, the use of EDGAR for core-genome-based phylogenomic/taxonomic analysis has become a main application field of the software. The web-based user interface of EDGAR offers all tools required for phylogenomic inter- and intraspecies taxonomic analyses as needed for the proposal of novel species. EDGAR calculates core-genome-based phylogenetic trees with neighbor-joining and maximum-likelihood methods, furthermore the genome taxonomy database (GTDB) phylogeny pipeline is currently added to the platform. As genome similarity indices, the classical amino acid identity (AAI) and average nucleotide identity (ANI) matrices are provided, fastANI and OrthoANI results are also implemented and will be added to the web interface in 2020. Thus, the software supports a quick survey of evolutionary relationships and simplifies the process of obtaining new biological insights into the differential gene content of kindred genomes.

EDGAR also provides 322 precomputed genus-based projects comprising 8,079 complete genomes providing comparative genomics and phylogenomic results. In addition, recently 226 new public projects were created that are clustered on the family level and exclusively use type strain genomes. These new projects comprise a further 4,400 genomes. EDGAR is free for academic use and funded as a service by the German Network for Bioinformatics Infrastructure – de.NBI. EDGAR is available via the public web server <http://edgar.computational.bio>.

---

### 365-PRP

#### Microbiological monitoring of thermoregulatory devices used for extracorporeal membrane oxygenation (ECMO): Is there a need for standards?

\*A. F. Wendel<sup>1</sup>, C. Karagiannidis<sup>2</sup>, F. Mattner<sup>1</sup>

<sup>1</sup>Cologne Merheim Medical Centre, University Hospital of Witten/Herdecke, Institute of Hygiene, Köln, Germany

<sup>2</sup>Cologne Merheim Medical Centre, University Hospital of Witten/Herdecke, Department of Pneumology and Critical Care Medicine, Köln, Germany

**Objectives:** Extracorporeal membrane oxygenation (ECMO) is a lifesaving technology for patients with severe respiratory failure. The thermoregulatory heater-cooler unit (HCU) of the medical device adjusts the blood temperature. The open system runs with circulating water. There is a risk of bacterial biofilm formation and subsequent transmission via aerosols or contact. The aim of this study was to assess this risk by microbiological sampling.

**Methods:** The study was conducted at a supra-regional ECMO centre in 2019. Water samples (800 ml) were taken weekly and aseptically from the water tanks, filled with sterile water, while the device was running. Total bacteria counts and *Legionella* spp. were analysed according to official recommendations. For mycobacterial investigations water samples were decontaminated, concentrated and plated on Middlebrook agar. Microscopy and identification using MALDI-TOF was performed from all grown colonies. Decontamination of the device was carried out according to the manufacturer's instructions.

**Results:** A total of 26 water samples from 6 different HCU (Maquet, Medos) were analysed. We did not detect any *Legionella* spp. or mycobacteria. One HCU yielded a total bacteria count >100 CFU/100ml (37°C). Interestingly, we observed growth of various nonfermenters (*Methylobacterium organophilum*, *Ralstonia insidiosa*, *Pseudomonas veronii*, *Cupriavidus pauculus*) in two HCU, even after disinfection. No transmissions were observed. Observations revealed possible contributing factors of contamination such as temporary storage near water sinks, disconnection of the tubing during patient transports or water refill.

**Conclusion:** There is a risk of biofilm formation of waterborne pathogens within the ECMO's HCU that can be highly resistant to disinfectants. However, the infection risk remains unclear. Microbiological monitoring standards have to be implemented, especially to control for contamination of leased devices.

---

### 366-PRP

Eye-catching microbes - Quantification and NGS-based identification of the bacteriota on microscope oculars

\*B. Fritz<sup>1</sup>, K. Schäfer<sup>1</sup>, F. Ziemssen<sup>2</sup>, S. Wahl<sup>3</sup>, M. Eger<sup>1</sup>

<sup>1</sup>Furtwangen University, Institute of Precision Medicine, Villingen-Schwenningen, Germany

<sup>2</sup>Eberhard Karls University, Centre for Ophthalmology, Tübingen, Germany

<sup>3</sup>Eberhard Karls University, ZEISS Vision Science Lab, Tübingen, Germany

**Introduction:** Regularly touched surfaces usually represent fomites, particularly if touched by different persons. This also applies for microscopes, widely used in clinical or biological laboratories. Previous, cultivation-based studies<sup>1</sup> suggested that direct contact with microscope eye-pieces significantly increases the risk of eye infections.

**Objectives:** In order to obtain a comprehensive view of the community profile and its hygienic relevance, we performed cultivation-based and molecular analyses of the ocular bacteriota.

**Material & Methods:** We swab-sampled oculars of 10 recently used microscopes from a university laboratory. Samples of the left oculars were used for quantification and MALDI-biotyping of representative isolates, following protocols previously established for spectacles<sup>2</sup>. Samples from the right oculars were used for a 16S rRNA gene sequencing approach<sup>3</sup>. After sampling, all oculars were carefully cleaned with isopropanol, re-sampled and analysed again.

**Results:** All oculars were contaminated with bacteria, with a maximum load of  $1.7 \times 10^3$  CFU cm<sup>-2</sup>. 114 morphologically different isolates were assigned to 64 genera and 34 species, mainly *Cutibacterium* (46%), *Staphylococcus* (21%) and *Brevibacterium* (5%), with the most abundant species being *Cutibacterium acnes* (13 %) and *Staphylococcus capitis* (6%). Cleaning reduced the microbial load up to 98%, leaving mainly cutibacteria. 15 samples yielded good sequencing results. 1517 ASVs were assigned to 10 phyla and 272 genera. The dominant genera before cleaning were *Cutibacterium* (78%), *Paracoccus* (13%), *Pseudomonas* (2%) and *Acinetobacter* (1%). The post-cleaning bacteriota composition was very similar, however, probably largely representing dead bacteria.

**Conclusion:** Here we report the first comprehensive insight into the microscope bacteriota. Used oculars were notably contaminated with skin and environmental bacteria, including potential pathogens. Regular cleaning after use is highly recommendable to prevent eye and skin infections.

<sup>1</sup>Olcerst (1987), Am Ind Hyg Assoc J. 48: 425-31.

<sup>2</sup>Fritz et al. (2018), PLoS ONE 13(11):e0207238

<sup>3</sup>Fritz et al. (2019), Sci Rep, submitted

### 367-PRP

Adaptation of *Listeria monocytogenes* to benzalkonium chloride induced viable but not culturable (VBNC) state

\*M. Noll<sup>1</sup>, K. Trunzer<sup>1</sup>, A. Vondran<sup>1</sup>, S. Vincze<sup>2</sup>, R. Dieckmann<sup>2</sup>, S. Al Dahouk<sup>2</sup>, C. Gold<sup>1</sup>

<sup>1</sup>Coburg University of Applied Sciences, Institute for Bioanalysis, Coburg, Germany

<sup>2</sup>German Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany

The objective of our study was to investigate the effects of benzalkonium chloride (BC) on the susceptibility of *L. monocytogenes* to antimicrobial agents (BC and antibiotics) and on the viable but not culturable (VBNC) state of the bacterial cells. We adapted *L. monocytogenes* by applying BC below minimum inhibitory concentration (MIC) to above minimum bactericidal concentration (MBC). The culturable fractions and the susceptibility of adapted and parental cells to BC were assessed. In addition, cell membrane permeability and glucose uptake were analyzed by multi parametric flow cytometry using the fluoresceins SYTO9, propidium iodide and 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxyglucose. Adapted cells displayed a two-fold MIC increase of BC and two additional antibiotic resistances while MBC remained the same. At high BC concentrations, the decrease in the number of colony forming units was significantly lower in the population of adapted cells than of parental cells. Growth-independent viability assays revealed an adapted subpopulation that did not grow on plates, but had an intact membrane and could maintain glucose uptake, indicating an increased abundance of VBNC cells. The observed data underline, that disinfectants may trigger antibiotic resistance and the VBNC state in *L.*

*monocytogenes* cells when inadequately applied. Standard culture techniques may fail to detect these adapted subpopulations on selective media. Moreover, adapted cells can outcompete non-adapted cells under sublethal concentrations of disinfectants, which may lead to novel public health risks.

### 368-PRP

Efficient detection and treatment of biofilms

\*G. Gotzmann<sup>1</sup>, D. Weile<sup>1</sup>, U. König<sup>1</sup>

<sup>1</sup>Fraunhofer FEP, Medical & Biotechnological Applications, Dresden, Germany

**Introduction** Biofilms raise problems in all areas of daily life. Especially in medicine there is a high risk of infections due to biofilm growth on implants and catheters.

**Objectives** There is no method to effectively prevented or inactivated biofilms. Our research aims for novel approaches for detection and inactivation of biofilms. The first step for an effective biofilm treatment is the fast detection of its growth. We aimed to have an in-situ device for real time detection. In a next step, bacteria shall be eliminated by inactivation and degradation of the organic residues.

**Materials&Methods** Typical biofilm contaminated objects are fingerprint sensors. Technological basis of such sensors are bidirectional displays. Such devices can emit light and detect reflected light in parallel, hence allowing a real time detection of biofilms on the surface. Inactivation and removal can be conducted by photocatalytic surface cleaning using special coatings made of TiO<sub>2</sub>. The combination of detection and cleaning is done by coating the surface of above mentioned displays with specially adapted layers. The effectiveness of such layers was investigated using the stearic acid degradation test, measurement of methylene blue reduction and inactivation of bacteria.

**Results** We found that biofilm detection on the surface is possible, independent from surrounding light, even detection in dark environment is possible. Furthermore, we were able to quantify the biofilm formation. Using the stearic acid test, we showed the degradation of surface organic contamination by photocatalysis. The same results were obtained for reduction of methylene blue in liquid environment and inactivation of *E.coli* as one example organism.

**Conclusion** The detection of contaminations even in dark surrounding make the application suitable also for pipes or also in the human body (implants, surgical instruments). The real time detection allows fast reaction on the biofilm formation and enables to treat them on demand and not "in advance". The specially developed titanium coatings applied on the surface of a bidirectional display permit a combination of contamination detection and "self cleaning".

### 369-PRP

The role of ions in photodynamic inactivation

\*D. B. Eckl<sup>1</sup>, L. Schottenhaml<sup>1</sup>, V. Mildenerger<sup>1</sup>, S. Eben<sup>1</sup>, A. Eichner<sup>2</sup>, R. Vashold<sup>3</sup>, W. Bäumler<sup>2</sup>, H. Huber<sup>1</sup>

<sup>1</sup>Universität Regensburg, Lehrstuhl für Mikrobiologie, Regensburg, Germany

<sup>2</sup>Universitätsklinikum Regensburg, Klinik und Poliklinik für Dermatologie, Regensburg, Germany

<sup>3</sup>Universität Regensburg, Institut für Organische Chemie, Regensburg, Germany



Photodynamic inactivation is a method allowing efficient killing of bacteria via reactive oxygen species by means of photosensitizers and visible light. Many reports on photodynamic inactivation show diverging results for the efficiency of the method. However, the reason for this remained unclear. Investigations on novel flavin based photosensitizers show that the efficiency of photodynamic inactivation depends on multiple factors such as the presence of ions. In the presented study, an interdisciplinary approach is used for clarifying this situation.

Prior before application of the photosensitizer to bacteria, the photosensitizers were incubated with various ions and spectroscopy was carried out in order to detect undesired chemical reactions. The altered structure of the photosensitizers was then analyzed via HPLC and LC-MS. Physical behavior of the photosensitizers in the presence of ions was checked with DPBF fluorescence measurements. As photosensitizers need to attach to bacterial cells to be efficient enough, attachment assays were performed where the amount of bound photosensitizer to the cells was measured. To complete the picture, bacterial cells were treated with different photosensitizer concentrations in combination with ions and other substances. After treatment, the cells were immediately transferred into fresh and pre-warmed medium. This procedure was followed up by the measurement of growth curves and subsequent calculation of logarithmic reduction.

Chemical analysis indicated that flavin based photosensitizers are heavily influenced by ions, which for example served in some other publications as buffer substances. Chemical alterations of the photosensitizers also affect the physical properties and the capability in producing singlet oxygen. For a variety of substances, no chemical or physical alteration was obvious. However, binding assays indicate a alteration in the attachment of the photosensitizer to the cells. Besides the negative side effects of photodynamic inactivation in chemical and physical manners, the data suggest that the outer structure of bacteria might play a crucial role in the efficiency of photodynamic inactivation.

### 370-PRP

Cold atmospheric-pressure plasma for the improvement of hygiene in medical or healthcare facilities

\*V. Hahn<sup>1</sup>, T. von Woedtke<sup>1</sup>, J. F. Kolb<sup>1</sup>, K. D. Weltmann<sup>1</sup>

<sup>1</sup>Leibniz Institute for Plasma Science and Technology (INP), Greifswald, Germany

**Introduction:** In Germany, nosocomial infections cause 400.000 - 600.000 cases of disease with 6.000 - 15.000 cases of death every year [1,2]. The usual disinfection and decontamination processes may be insufficient for particular applications such as air hygiene, treatment of wastewater or processing of medical instruments with sensitive materials or small cavities.

**Objectives:** Physical plasma can be established as an alternative method for standard processes such as the decontamination of surfaces (materials, devices), liquids/water or aerosols/gases.

**Materials and Methods:** Cold or non-thermal plasma (room temperature) can be generated artificially and exploited for an effective decontamination. Different plasma sources are conceivable depending on treated goods and objective. Two examples are "pin-to-liquid" discharges for a treatment of liquids or dielectric barrier discharges for the treatment of large surfaces or air.

**Results:** The biological decontamination comprises the plasma-mediated inactivation of microorganisms including multidrug-resistant strains. Furthermore, a chemical decontamination is possible such as the elimination of toxic or unwanted pollutants (including odours and pharmaceutical residues).

**Conclusion:** Plasma technologies offer different advantageous properties. Cold plasma avoids high temperatures (below 40°C), pressures or disinfectants with high alcohol content. Thus, plasma is in particular suitable for the treatment of sensible goods such as heat sensitive materials or objects with complex shapes. In addition, as an essentially chemical-free physical process, plasma is especially suitable for the use in medical and healthcare facilities.

[1]

[https://www.rki.de/SharedDocs/FAQ/Krankenhausinfektionen-und-Antibiotikaresistenz/FAQ\\_Liste.html#FAQId5983052](https://www.rki.de/SharedDocs/FAQ/Krankenhausinfektionen-und-Antibiotikaresistenz/FAQ_Liste.html#FAQId5983052) (state: 16.11.2018)

[2] <https://www.aerzteblatt.de/nachrichten/99656/Bis-zu-600-000-Krankenhausinfektionen> (state: 06.12.2018)

### 371-PRP

**Development of a flexible software solution to document and supervise flushing of drinking water pipelines in the hospital environment**

\*T. Schultze<sup>1,2</sup>, D. Bergmann<sup>1,2</sup>, V. A. J. Kempf<sup>1,2</sup>

<sup>1</sup>Institute for Medical Microbiology and Infection Control, University Hospital, Goethe University Frankfurt am Main, Frankfurt am Main, Germany

<sup>2</sup>University Center of Competence for Infection Control, State of Hesse, Frankfurt am Main, Germany

**Introduction:** Water in rarely used drinking water systems represents a potential source of bacterial contamination as it might become a reservoir for biofilm-forming bacterial species such as *Legionella* or *Pseudomonas*. Human infections with these bacteria harbour a life-threatening risk and have to be prevented especially in a hospital environment. On a legal basis, countermeasures are required to comply with the German "Infektionsschutzgesetz" (IfSG) and the "Trinkwasserverordnung" (TrinkwV).

Currently, routinely flushing of pipelines at risk is a state of the art solution to overcome this problem. However, for large facilities this results in logistical challenges of documentation supervising this process.

**Objective:** Development of a software tool which simplifies and standardizes the documentation process granting supervisors and authorities immediate access to these reports.

**Material & Methods:** To this end, a protected Microsoft Excel based project was created utilizing VBA ActiveX-controls.

**Results:** The first mode of the software is the administrative mode which allows creating or managing of documentation templates. The second mode is the working mode which utilizes these templates for the reoccurring documentation process. As this software runs on a mobile device such as a laptop or surface laptop, the technical staff can directly document the process while flushing the pipelines. Moreover, besides the printing of reports for paper-based documentation, the software also creates an electronic report

on an archive path thereby enabling direct supervision of the process.

**Conclusion:** We present a software solution simplifying the documentation of the flushing manouvers of drinking water pipelines.

---

### 372-PRP

Microorganisms living in water over a prolonged time process a significant increase of tolerance to disinfections

\*M. Hummert<sup>1</sup>, O. Böhler<sup>1</sup>, T. Kuczius<sup>1</sup>

<sup>1</sup>Institute of Hygiene, University of Münster, University Hospital of Münster, Münster, Germany

The contamination of water sources with pathogenic and drug-resistant microorganisms is considered a major public health problem. Drinking water quality is controlled by cultivation on selective nutrients at which only planktonic and viable bacteria are verifiable. However, most of the bacteria live in biofilms or survive in an inactive state as viable-but-non-cultivable (VBNC) bacteria. Isolates cultivated in rich nutrient media are highly susceptible to disinfections and antibiotic treatments. In contrast, bacteria living in water without any additional nutrient supply demonstrate an increased tolerance to disinfections. In this study we adapted microorganisms to drinking water and followed the survival and increase of tolerance to disinfection during long term incubation. *Pseudomonas aeruginosa* and *Escherichia coli* which are human-pathogens were cultivated for more than 12 weeks under low shaking conditions to avoid biofilm formation. At distinct time periods isolates were exposed to disinfection procedures with chlorine (1.2 mg/L) and hydrogen peroxide (50 mg/L) and to antibiotic treatment.

Bacteria were susceptible to the disinfections within the first two weeks in drinking water while the tolerance increased highly over the time. This increase of tolerance is accompanied with a process of changes in bacterial physiology followed by a dormancy state with less ATP production and changes in signals detected by flow cytometry. Our results indicate long term survival of microorganisms in water sources over the time which is accompanied with significant physiological changes and high tolerance to disinfections. Periodic changes of disinfection agents may be an option to minimize such an increase in bacterial tolerances.

---

### 373-PRP

#### Disinfection of dry inanimate surfaces in complex room conditions using ozone whole room disinfection

B. Knobling<sup>1</sup>, C. E. Belmar Campos<sup>1</sup>, E. M. Klupp<sup>1</sup>, G. Franke<sup>1</sup>, \*J. K. Knobloch<sup>1</sup>

<sup>1</sup>University Medical Center Hamburg Eppendorf, Institute for Medical Microbiology, Virology and Hygiene, Hamburg, Germany

**Introduction** Several pathogenic bacterial species can display a high ability to survive on inanimate surfaces. Therefore, cleaning and disinfection is an important part in the prevention of transmission of these organisms. No-touch room decontamination is performed with increasing frequency to reach more standardization in hospital cleaning. In this study, we investigated the efficacy of an automated room decontamination using ozone against *A. baumannii* and *E. faecium* under complex room conditions.

**Material & Methods** A Carbapenemase-producing *A. baumannii* outbreak strain and *E. faecium* ATCC 6057 were analyzed with respect to their sensitivity against ozone. The

Sterisafe™ pro instrument was used in a patient room with an attached bathroom. The strains were dried on three different carriers (ceramic tiles, stainless steel, solid core furniture board) and placed at eight different positions in the room. A standard disinfection cycle (80 ppm ozone; 90 % RH; 60 min) was conducted in three independent experiments. Quantitative culture was performed from treated and untreated surfaces with subsequent calculation of the relative reduction rate (log<sub>10</sub>).

**Results** In desiccation experiments *A. baumannii* as well as *E. faecium* ATCC 6057 displayed a long term survival on surfaces under dry conditions sufficient for disinfection experiments. Using the standard disinfection cycle of the Sterisafe™ pro instrument mean reduction rates greater than 5 log were reached under the complex room conditions. No influence of the position of the carriers was observed, indicating that ozone reach all surfaces within the room and side rooms with opened doors. However, slight but reproducible differences were observed for the reduction rate on different types of surface.

**Conclusions** The Sterisafe™ pro instrument displayed a sufficient reduction of *A. baumannii* *E. faecium* ATCC 6057 even under complex room conditions. The behavior of bacteria on different materials should be investigated in more detail in the future to validate automated room decontamination systems under varying conditions.

---

### 374-PRP

Antibacterial activity of nanostructured Zinc oxide tetrapods

\*A. Bütter<sup>1</sup>, G. Maschkowitz<sup>1</sup>, M. Baum<sup>2</sup>, Y. Mishra<sup>3</sup>, R. Adelung<sup>2</sup>, H. Fickenscher<sup>1</sup>

<sup>1</sup>Institute for Infection Medicine, Kiel, Germany

<sup>2</sup>Institute for Material Sciences, Department for Functional Nanomaterials, Kiel, Germany

<sup>3</sup>University of Southern Denmark, Mads Clausen Institute, Sønderborg, Denmark

Zinc oxid (ZnO) tetrapods are microparticles with nanostructured surfaces showing specific physical properties, which can be chemically modified. In comparison to globular unstructured ZnO, tetrapods show a lower toxicity in eukaryotic cell culture. Previously, antiviral activities of ZnO tetrapods against herpes simplex virus types 1 and 2 were described. Since ZnO tetrapods are non-soluble particles, they need to be applied in suspension.

The aim of this study was to investigate the antibacterial properties of ZnO tetrapods in comparison to commercially available globular ZnO. Killing rates of tetrapods and globular ZnO against Gram-negative and Gram-positive bacteria were determined for different time points and for different concentrations.

While *Staphylococcus aureus* strains showed a cell-number reduction in average of 3 log<sub>10</sub> levels at 1 mg/ml after 24 hours of incubation, *Klebsiella pneumoniae* isolates showed a similar cell-number reduction with 2 mg/ml, only. However, ZnO tetrapods did not show effects on *Pseudomonas aeruginosa* and *Enterococcus faecalis* strains.

Thus, ZnO tetrapods show antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* isolates. Surface modifications of the ZnO tetrapods might allow alterations of the host range of antibacterial activity. ZnO tetrapods add an additional principle to the spectrum of antibacterial mechanism which is independent of antibiotics.

---

### 375-MPP

#### **Pro-thrombotic and pro-inflammatory activities of the $\beta$ -hemolytic group B streptococcal pigment**

H. F. Wunderink<sup>1</sup>, S. Oehmcke-Hecht<sup>2</sup>, J. Hoßmann<sup>3</sup>, S. B. Skorka<sup>4</sup>, R. H. T. Nijhuis<sup>5</sup>, C. Ruppen<sup>6</sup>, S. Skrede<sup>7</sup>, M. Rohde<sup>3</sup>, D. Schultz<sup>8</sup>, M. Lalk<sup>8</sup>, A. Itzek<sup>3</sup>, D. H. Pieper<sup>3</sup>, C. J. van den Bout<sup>1</sup>, E. C. J. Claas<sup>9</sup>, E. J. Kuijper<sup>9</sup>, R. Mauritz<sup>9</sup>, P. Sendi<sup>6</sup>, A. Norrby-Teglund<sup>6,10</sup>, \*N. Siemens<sup>4</sup>

<sup>1</sup>University Medical Center Utrecht, Utrecht, Netherlands

<sup>2</sup>University Medicine Rostock, Rostock, Germany

<sup>3</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

<sup>4</sup>University of Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

<sup>5</sup>Meander Medical Center, Amersfoort, Netherlands

<sup>6</sup>University of Bern, Bern, Switzerland

<sup>7</sup>Haukeland University Hospital, Bergen, Norway

<sup>8</sup>University of Greifswald, Greifswald, Germany

<sup>9</sup>Leiden University Medical Center, Leiden, Netherlands

<sup>10</sup>Karolinska Institutet, Center for Infectious Medicine, Stockholm, Sweden

**Introduction.** A prominent feature of severe streptococcal infections is the profound inflammatory response that contributes to systemic toxicity. In sepsis the dysregulated host response involves both immunological and non-immunological pathways.

**Objectives.** The aim of the study was to analyze the impact of group B streptococcal pigmentation in relation to systemic toxicity.

**Methods.** A series of molecular, biochemical, and immunological assays was performed.

**Results.** Here, we report a fatal case of an immunocompetent healthy female presenting with toxic shock and *purpura fulminans* caused by group B streptococcus (GBS). The strain was pigmented and hyper-hemolytic. Stimulations of human primary cells with hyper-hemolytic GBS strains and pigment toxin resulted in a release of pro-inflammatory mediators, including TNF, IL-1 $\beta$ , and IL-6. In addition, pigmented GBS induced blood clotting and showed FXII activity on their surfaces, which was linked to the presence of the pigment.

**Conclusion.** Detailed characterization of the pigmented GBS strains coupled to functional studies implicated the pigment as a key factor eliciting pro-thrombotic and inflammatory responses.

manifestation and progression, which includes pneumonia, meningitis, and sepsis. Among them, pneumolysin is believed to be one of the major pneumococcal virulence factors. It induces pore-formation and subsequent lysis of host cells via receptor-independent mechanisms. However, neutrophil activation and degranulation in response to pneumolysin are relatively unexplored.

### **Objectives**

Here, we investigated the degree and specificity of pneumolysin-mediated activation of human primary neutrophils.

### **Material & methods**

Primary human neutrophils were exposed to the pneumococcal strains TIGR4 and its Ply deficient mutant or directly to different concentrations of purified pneumolysin. Cytotoxicity was measured and pore formation was assessed via scanning electron microscopy. Neutrophil activation was assessed by measurement of secreted azurophilic granule protein resistin and determination of the whole secretome, using mass spectrometry.

### **Results**

We show that even at sub-lytic concentration Ply is a potent inducer of activation and degranulation. In contrast to previous findings, it is a purine type II X7 receptor (P2RX7)-independent mechanism. However, P2RX7 agonist adenosine triphosphate (ATP) almost completely abolished pneumolysin-mediated neutrophil lysis and degranulation. Microscale thermophoresis revealed that ATP binds to pneumolysin in mM range, thereby impairing pneumolysin-neutrophil interactions.

### **Conclusion**

We conclude that pneumolysin is a potent major pneumococcal activator of neutrophils. However, ATP binds to pneumolysin and subsequently neutralizes pneumolysin-mediated effects. The observed phenomenon is P2RX7-independent. Our data further indicate that ATP might serve as a crucial sensor of bacterial components. This observation warrants further studies.

### 376-MPP

#### **Extracellular Adenosine Triphosphate Neutralizes Pneumolysin-induced Neutrophil Lysis and Degranulation**

\*F. Cuyper<sup>1</sup>, B. Klabunde<sup>1</sup>, M. Rohde<sup>2</sup>, M. Gesell Salazar<sup>3</sup>, S. Skorka<sup>1</sup>, S. Surabhi<sup>1</sup>, T. Thiele<sup>4</sup>, U. Völker<sup>3</sup>, K. Study Group<sup>1</sup>, S. Hammerschmidt<sup>1</sup>, N. Siemens<sup>1</sup>

<sup>1</sup>University of Greifswald, Interfaculty Institute of Genetics and Functional Genomics, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

<sup>2</sup>HZI - Helmholtz Centre for Infection Research, Central Facility for Microscopy, Braunschweig, Germany

<sup>3</sup>University of Greifswald, Center for Functional Genomics of Microbes, Department of Functional Genomics, Greifswald, Germany

<sup>4</sup>Institute for Immunology and Transfusion Medicine, Greifswald, Germany

### **Introduction**

Neutrophil migration to the site of infection is a crucial step in host defense. *Streptococcus pneumoniae* is equipped with an arsenal of virulence factors responsible for disease

### 377-MPP

#### **A battle for survival – the interplay between autophagy and *S. aureus* infection**

\*A. Riebesch<sup>1,2</sup>, Y. Neumann<sup>1,2</sup>, S. Bruns<sup>1,2</sup>, A. Kiesewetter<sup>1</sup>, I. Schmitz<sup>1,2</sup>

<sup>1</sup>Helmholtz Centre for Infection Research, Systems-oriented Immunology and Inflammation Research Group, Braunschweig, Germany

<sup>2</sup>Otto von Guericke University, Institute for Molecular and Clinical Immunology, Magdeburg, Germany

The human pathogen *Staphylococcus aureus* is the leading cause of hospital acquired infections and its multidrug resistance makes a successful treatment more and more difficult. Originally, *S. aureus* was considered to be an extracellular bacterium, but it has now been shown that it can invade a variety of eukaryotic cells, where it must evade the intracellular defence mechanisms to survive.

One defence mechanism against intracellular bacteria is xenophagy, a specialised form of autophagy. Autophagy is a highly conserved catabolic process that degrades

cytoplasmic contents through the lysosomal pathway and acts not only as an efficient recycle and survival mechanism during cellular stress, but also as an anti-infective machinery.

In 2016, our group showed for the first time the induction of selective autophagy by *S. aureus* infection, its escape from the autophagosome and proliferation in the cytoplasm, via live cell imaging. After invasion, *S. aureus* co-localises with ubiquitin and the receptor proteins SQSTM1, OPTN and CALCOCO2, leading to phagophore recruitment. Additionally, p38 MAPK activation was observed, which is a so far unrecognised bacterial strategy to manipulate the autophagic pathway.

The activation of the p38 MAPK could be due to either the bacteria triggering a host cell receptor or by direct activation by bacterial effector proteins. Here, we analyse the impact of Nod and Toll-like receptors on MAPK activation and autophagy induction during *S. aureus* infection. Additionally, bacterial and host cell signal transducers will be analysed regarding their impact on p38 MAPK and autophagy induction. Surprisingly, the *S. aureus* surface expressed kinase PknB, which has as MAP Kinase-like activity, is not involved in p38 MAPK activation and autophagy induction. Due to this observation, we are performing a global transcriptome analysis to identify proteins that might be relevant for the inhibition of the fusion of the autophagosome and the lysosome as well as the escape of *S. aureus* of the autophagosome.

A further understanding of the molecular mechanisms of this host-pathogen interaction will facilitate new strategies to combat this important pathogen.

---

### 378-MPP

#### Impact of extracellular pneumococcal serine proteases on pneumococcal phagocytosis and virulence

\*M. Q. Ali<sup>1</sup>, N. Mehltitz<sup>1</sup>, A. Wüst<sup>1</sup>, R. Bolsmann<sup>1</sup>, T. P. Kohler<sup>1</sup>, G. Burchhardt<sup>1</sup>, S. Hammerschmidt<sup>1</sup>

<sup>1</sup>Center for Functional Genomics of Microbes, Department of Molecular Genetics and Infection Biology, University of Greifswald, Greifswald, Germany

Introduction and objectives: *Streptococcus pneumoniae* (the pneumococcus) asymptotically colonize mucosal surfaces of healthy human individuals. Due to its high virulence potential, pneumococci can cause pneumonia, sepsis and meningitis. Four serine proteases (HtrA, PrtA, Sfp, and CbpG) are expressed by *S. pneumoniae* and surface-exposed. The impact on pneumococcal pneumonia of the individual serine proteases was low. To analyse the pathological effects caused by pneumococcal serine proteases in a more systematic approach, we generated double and triple serine protease deficient strains. Methods: We generated triple serine protease mutants, with only one functional enzyme present, by insertion-deletion mutagenesis in nonencapsulated TIGR4Δcps and encapsulated TIGR4lux to perform phagocytosis assays and in vivo infections. We infected mouse macrophages J774A.1 with strain TIGR4Δcps or isogenic serine protease mutants and enumerated the number of internalized and recovered pneumococci. In addition, to visualize the extra and intracellular pneumococci, we conducted immunofluorescence microscopy. For the acute pneumonia mouse model, mice we intranasally infected mice with TIGR4lux expressing only one serine protease. To visualize the dissemination of pneumococci from the nasopharynx to the lung and the blood, we monitored the spread of pneumococci using the IVIS® Spectrum bioimaging system at different time points. Results: The infection of macrophages resulted in a significant reduction in number of

recovered triple serine protease knockout mutants compared to the wild-type strain. Furthermore, the enumeration of triple mutants internalized by macrophages was also reduced. Intranasal infection of mice showed only an attenuation and extended survival with the triple mutant, in which only the immunogenic PrtA is expressed. Conclusion: Interestingly, loss of the functional serine proteases showed a lower internalization of *S. pneumoniae* by macrophage. The results of the acute pneumococcal pneumonia model suggest that even the presence of one serine protease is sufficient to cause dramatic lungs infections and septicemia. Further studies will show the impact of serine proteases on long-term colonization and mild lung infections.

---

### 379-MPP

#### Analysis of the RNA-based regulatory mechanisms controlling virulence in *Yersinia pseudotuberculosis*

\*I. Vollmer<sup>1</sup>, M. Kusmirek<sup>2</sup>, A. K. Heroven<sup>2</sup>, P. Dersch<sup>1</sup>

<sup>1</sup>Zentrum für Molekularbiologie der Entzündung, Institut für Infektiologie, Münster, Germany

<sup>2</sup>Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany

The human pathogen *Yersinia pseudotuberculosis* is able to adhere to the epithelial cell layer and evade the host immune response after passage through the M-cells of the small intestine, allowing the pathogen to replicate in deeper tissues and cause disease.

Virulence regulation in *Yersinia* is strictly controlled and influenced by numerous environmental factors such as an increase in temperature from 25°C to 37°C or contact to host cells. This ensures an organised expression of virulence factors necessary for infection establishment. Among the virulence proteins are those involved in the initial infection phase, such as adhesins and flagella, but also proteins of the on-going infection including the type III secretion system and its secreted Yop effector proteins encoded on the virulence plasmid pYV. Many of these virulence factors are under the control of the plasmid-encoded transcriptional regulator LcrF, whose 5'-UTR undergoes a temperature-dependent opening of a thermo-loop, leading to efficient translation of the *lcrF* mRNA.

RNases play an important role in a variety of cellular processes. Among them are the endo- and exoribonucleases RNaseE and PNPase, which are part of a multi-enzyme complex called the degradosome, and RNaseIII. Recent data from our lab suggests that these RNases are involved in the regulation of different virulence traits such as type III and type VI secretion and invasion. These roles are currently under further investigation, for example by RNAseq. This will give us an important insight into the versatile role of RNases in the infection process of this enteric pathogen.

---

### 380-MPP

#### The role of *VraSR* on the reduced susceptibility to vancomycin in a laboratory VISA strain

\*M. Hort<sup>1</sup>, A. S. Schrötter<sup>1</sup>, A. Berscheid<sup>2</sup>, U. Bertsche<sup>3</sup>, G. Bierbaum<sup>1</sup>

<sup>1</sup>University Hospital Bonn, Institute of Medical Microbiology, Immunology and Parasitology, Bonn, Germany

<sup>2</sup>University of Tübingen, Interfaculty Institute for Microbiology and Infection Medicine, Tübingen, Germany

<sup>3</sup>University of Hohenheim, Mass Spectrometry Unit, Stuttgart, Germany

Vancomycin is the drug of choice for many severe MRSA infections. However, in the past 20 years many clinical isolates with reduced susceptibility to Vancomycin, called

vancomycin-intermediate susceptible *S. aureus* (VISA), have been isolated. Common features of VISA strains comprise decreased virulence, reduced autolysis and a thickened cell wall with low peptidoglycan crosslinking [1]. Here we focus on the important two-component system (TCS) *VraSR* of a laboratory VISA strain *S. aureus* VC40 (MIC 64 µg/mL) [2]. The strain was generated by serial passage of a mutator strain *S. aureus* RN4220  $\Delta$ *mutS* (MIC 2 µg/mL) in the presence of vancomycin. It had accumulated a total of 79 mutations, two of these leading to exchanges in *VraS* (D242G; L114S) [3]. The *VraSR* TCS is known to regulate gene expression in response to cell wall active antibiotics. Also a revertant was isolated with a reduced vancomycin resistance (MIC 4 µg/mL) and an additional mutation in *vraR* (M54T), labelled SA Revertant. Additionally, the two mutations of *vraS* were introduced into the *S. aureus* NCTC8325 background (MIC 4 µg/mL) [3]. Using microarray and qRT-PCR, it could be shown that the two mutations in *vraS* led to an upregulation of the *vraSR* regulon [3]. In contrast, the mutation in *vraR* of the SA Revertant strain reduced transcription of the *vraS* gene to the level of the WT control. However, the mutated *VraR* (M54T) protein of the SA Revertant strain showed a slower migration in the Phos-tag SDS-PAGE, which might indicate phosphorylation. Yet this could not be confirmed by mass spectrometry. The *vraS* mutations seem to lead to a reduced autolysis, possibly due to a reduced binding and an altered processing of *AtlA*, the major autolysin in *S. aureus*. The reduced autolysis also coincided with a difference in wall teichoic acid composition in SA VC40 and the strain harbouring the *VraS* mutations. In conclusion, these results indicate that *VraSR* influences autolysis by a so far uncharacterized mechanism.

[1]Hu *et al.* 2016 *Front. Microbiol.* 7:1601; [2] Schaaff *et al.* 2002 *Antimicrob. Agents Chemother.* 46(11):3540-8; [3] Berscheid *et al.* 2014 *J. Antimicrob. Chemother.* 69(12):3190-8

### 381-MPP

#### Validation of a novel drug target in *Mycobacterium tuberculosis*

\*S. Krysenko<sup>1</sup>, M. Oswald<sup>1</sup>, A. Kulik<sup>1</sup>, W. Wohlleben<sup>1</sup>, A. Bera<sup>1</sup>  
<sup>1</sup>University of Tuebingen, IMIT, Microbiology and Biotechnology, Tuebingen, Germany

Human intracellular pathogenic actinobacterium *Mycobacterium tuberculosis* has developed strategies to access nutrients from the host and to exploit the host to synthesize more resources for its growth and propagation. *Mycobacterium tuberculosis* can induce the polyamine biosynthesis during the shift in metabolic state of macrophages. The pathogen is able to utilize polyamines as a sole N- and C-source to support its own intracellular growth in macrophages. In our previous studies in a model actinobacterium *Streptomyces coelicolor* M145, which possesses three glutamine synthetase (GS)-like enzymes, we demonstrated that only one GS-like protein GlnA3St (SCO6962), is involved in the first step of polyamine utilization pathway [1],[2]. GlnA3St is a gamma-glutamylpolyamine synthetase (GPS) that ensures both nutrients availability (C- and N-source) and resistance against high polyamine concentrations in *Streptomyces coelicolor* [1]. Since there is a homologue of GlnA3Mt (Rv1878) in *Mycobacterium tuberculosis*, this GPS enzyme is a particularly interesting target for drug development. In our current studies we were able to show that GlnA3Mt can glutamylate polyamines, demonstrating GPS activity [3]. Thus, GlnA3Mt is a novel specific drug target in a relevant human pathogen.

1. Krysenko S., Okoniewski N., Kulik A., Matthews A., Grimpo J., Wohlleben W. and Bera A. (2017) Gamma-Glutamylpolyamine Synthetase GlnA3 Is involved in the first step of polyamine degradation pathway in *Streptomyces coelicolor* M145. *Front Microbiol* 8: 726.

2. Krysenko S., Matthews A., Okoniewski N., Kulik A., Girbas M. G., Tsyplik O., Meyners C. S., Hausch F., Wohlleben W., Bera A. (2019) Initial metabolic step of a novel ethanolamine utilization pathway and its regulation in *Streptomyces coelicolor* M145. *mBio* 10(3).

3. Krysenko S., Oswald M., Meyners C., Kulik A., Reiling N., Hilemann D., Paudyal B., Purder P., Hausch F. and Brötz-Oesterhelt H., Wohlleben W., Bera A. (2019) The  $\gamma$ -glutamylspermine synthetase GlnA3Mt is involved in the polyamine glutamylation in *Mycobacterium tuberculosis* (in preparation).

### 382-MPP

#### High nuclease activity of long persisting *Staphylococcus aureus* isolates confers protection against NET-mediated killing within the airways of CF patients

\*S. Herzog<sup>1</sup>, F. Dach<sup>1</sup>, C. Neumann<sup>1</sup>, S. Niemann<sup>1</sup>, D. Chaves-Moreno<sup>2</sup>, B. C. Kahl<sup>1</sup>

<sup>1</sup>Institut für Medizinische Mikrobiologie, Münster, Germany

<sup>2</sup>Helmholtz Association of German Research Centers (HZ), Helmholtz Center for Infection Research, Brunswick, Germany

*Staphylococcus aureus* is one of the first most prevalent pathogens in cystic fibrosis (CF). Airway infections in CF are characterized by a strong inflammatory response of highly recruited neutrophils. One killing mechanism of neutrophils is the formation of neutrophil extracellular traps (NETs) consisting of extracellular DNA fibers with antimicrobial granule proteins, which can be degraded by *S. aureus* nuclease.

We hypothesize, that *S. aureus* adapts to the airways of CF patients during persistent infection by escaping from NET-mediated killing via an increase of nuclease activity.

Sputum samples of CF patients were visualized by confocal microscopy after immuno-fluorescence staining. Nuclease activity was analyzed in long persisting *S. aureus* isolates from an individual CF patient, as well as from 29 different CF patients with varying *S. aureus* persistence, using a FRET based nuclease activity assay. Clinical isolates were further selected for qRT-PCR to determine expression of *nuc1* and *nuc2*. NET-killing assays were performed to evaluate bacterial survival depending on nuclease activity. To confirm the role of nuclease during NET-mediated killing, a clinical isolate with low nuclease activity was transformed with a nuclease expression vector (pCM28nuc). Two sputa from an individual CF patient were subjected to RNA-sequence analysis to evaluate the activity of nuclease in vivo.

In sputa, *S. aureus* was associated to extracellular DNA structures. Nuclease activity in clinical *S. aureus* isolates increased in a time- and phenotype-dependent manner. NET-mediated killing was significantly higher in *S. aureus* isolates with low compared to isolates with high nuclease activity. Transformation of the clinical isolate with low nuclease activity conferred protection against NET-mediated killing confirming the beneficial role of nuclease against NETs. Nuclease expression in in vivo sputa was high, which underlines the important role of nuclease within the highly inflamed CF airways.

In conclusion, our data show that *S. aureus* adapts to the neutrophil-rich environment of CF airways with increasing nuclease secretion most likely to avoid NET-killing during long-term persistence.

---

### 383-MPP

#### Life After Secretion—*Yersinia enterocolitica* Rapidly Toggles Effector Secretion and Can Resume Cell Division in Response to Changing External Conditions

\*B. Milne-Davies<sup>1</sup>, C. Helbig<sup>1</sup>, S. Wimmi<sup>1</sup>, D. W. C. Cheng<sup>2</sup>, N.

Paczia<sup>1</sup>, A. Diepold<sup>1</sup>

<sup>1</sup>Max Planck Institute for Terrestrial Microbiology, Ecophysiology, Marburg, Germany

<sup>2</sup>Reed College, Chemistry, Portland, United States

**Introduction:** The type III secretion system (T3SS) injectisome is a virulence mechanism that many pathogens utilize during infection. This system allows bacteria to manipulate the host to further allow dissemination. Secretion of effector proteins is accompanied by inhibition of growth; hence, many pathogenic species are heterogeneous for T3SS expression, which allows T3SS-inactive cells to thrive in the host and outcompete the T3SS-active cells and other competitors. In contrast, *Yersinia enterocolitica* consists of a homogeneous T3SS-active population. This homogenous behavior brings to question how *Y. enterocolitica* mediates successful infection within the host.

**Objective:** The aim of the study was to elucidate how *Y. enterocolitica* initiate and stop secretion, to describe their life after secretion, and to determine the link between growth arrest and secretion.

**Material & Methods:** Secretion kinetics upon activation and deactivation were measured using a sensitive quantitative enzymatic secretion assay. Survival and restart of growth and division following secretion was determined in different conditions. Metabolomics experiments were performed to uncover the molecular basis of the observed phenotypes.

**Results:** The results show that *Y. enterocolitica* deactivates the T3SS immediately in the absence of activating factors, resumes division within a short time, and is able to reactivate secretion using the same T3SS machinery. This fast response is not caused by energy depletion.

**Conclusion:** Our results demonstrate that *Y. enterocolitica* can respond rapidly to different environmental signals and can survive secretion. This suggests that unlike other pathogens that altruistically sacrifice themselves for the whole population, *Y. enterocolitica* behaves in a more individualistic manner to allow successful infection (Milne-Davies *et al*, 2019).

Milne-Davies B, Helbig C, Wimmi S, Cheng DWC, Paczia N & Diepold A (2019) Life After Secretion—*Yersinia enterocolitica* Rapidly Toggles Effector Secretion and Can Resume Cell Division in Response to Changing External Conditions. *Front. Microbiol.* 10:2128.

---

### 384-MPP

Unraveling the protein machinery and protein-interaction dynamics essential for flagellar and vesicle formation of *Pseudomonas aeruginosa*

\*J. M. Borrero de Acuna<sup>1</sup>, D. Jahn<sup>1</sup>, G. Molinari<sup>2</sup>, L. Jänsch<sup>2</sup>, J. Wissing<sup>2</sup>, M. Rohde<sup>2</sup>, K. Müller<sup>1</sup>

<sup>1</sup>Technical University Braunschweig, Microbiology, Braunschweig, Germany

<sup>2</sup>Helmholtz Zentrum für Infektionsforschung, Braunschweig, Germany

*Pseudomonas aeruginosa* is an opportunistic pathogen that colonizes the lung of immunocompromised patients such as those suffering from cystic fibrosis. The decreasing O<sub>2</sub> gradient generated as a result of the thick mucosa of a cystic fibrosis transmembrane conductance regulator mutated CF lung and the biofilm density poses an adverse scenario to bacteria that *P. aeruginosa* circumvents by its versatile metabolism, which encompasses nitrate/nitrite respiration, arginine and pyruvate fermentation as predominant energy generating routes. On the other hand, a successful colonization of the human host and penetration through the thick mucus extensively depends on the construction of a highly developed flagellar machinery, which endows this monoflagellated bacterium with both motility and adhesion capacities. In a previous work our group revealed that FliC, although quantitatively scarce, was distributed alongside the extracytosolic region and not exclusively located at the pole where the sole flagellum of *P. aeruginosa* is constructed. The periplasmic location of FliC was discovered to take place in a triad-manner with two other proteins, the periplasmic nitrite reductase involved in denitrification and the presumably cytosolic ATP-dependent molecular chaperone DnaK. Beyond identifying the interlaced function of the nitrite reductase in swimming motility and flagellar assembly no further characterization of a potentially alternative role of the DnaK besides its well-understood chaperone-like function in the flagellar export, construction or functionality had been pursued. In a recent study we monitored the location of DnaK and FliC throughout the different fractions and organelles of *P. aeruginosa* by harnessing scanning and transmission electron microscopy (S/TEM) coupled with single and double immunogold labelling, confocal microscopy and immunofluorescence and mass spectrometry (LC-MS/MS). Moreover, we deepened into the interactions of DnaK-FliC by employing SPOT-membrane arrays pinpointing the interacting domains of both proteins leading to ultimately infer a docking model. Finally, we link DnaK trafficking with flagellar movement and vesicle formation.

---

### 385-MPP

The non-phosphorylating glyceraldehyde 3-phosphate dehydrogenase GapN is the major source for NADPH and a potential new drug target in *Streptococcus pyogenes*

\*P. Eisenberg<sup>1</sup>, C. Michaelis<sup>1</sup>, C. Sehlke<sup>1</sup>, B. Kreikemeyer<sup>1</sup>, T. Fiedler<sup>1</sup>

<sup>1</sup>Universitätsmedizin Rostock, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Rostock, Germany

### Question

*Streptococcus pyogenes* (group A streptococcus, GAS) is an important Gram-positive pathogen causing local infections but also invasive or systemic diseases. The standard therapy of GAS infections is antibiotic treatment with penicillin, cephalosporine or macrolides. Allergies of patients to penicillin and resistances of GAS strains to macrolide antibiotics frequently occur and the mentioned antibiotics affect a broad range of bacteria of the physiological flora. New therapeutic agents should be more specific for the pathogen. Targets of new antimicrobials should be metabolic processes not present or not essential in human cells and in the majority of the physiological flora. The non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase GapN is a potential target. GapN converts glyceraldehyde 3-phosphate to 3-phosphoglycerate and generates NADPH. We hypothesize that this reaction is the major source for NADPH and therefore essential in GAS and

other bacteria lacking the oxidative part of the pentose phosphate pathway (oPPP). It might therefore be suitable as a drug target.

## Methods

Translation of *gapN* was inhibited with antisense peptide nucleic acids (asPNA) targeting the start codon region of *gapN* in kill assays with GAS M49 strain 591, 8 macrolide resistant clinical GAS strains, *S. salivarius*, *S. cristatus*, *S. equisimilis* and *S. pneumoniae*. GapN activity was measured via photometric detection of NADP reduction. GapN of GAS M49 591 was produced in *E. coli* using a pASK-IBA6 based plasmid and purified by StrepTactin affinity chromatography.

## Results

The GapN activity is significantly reduced in asPNA treated GAS M49 confirming the specific effect of the asPNA. GAS strains as well as *S. salivarius* and *S. equisimilis* (all oPPP-) were efficiently killed upon *gapN*-specific asPNA treatment while *S. pneumoniae* and *S. cristatus* (oPPP+) were less prone to gapN asPNA treatment. Furthermore, GAS-GapN was expressed and isolated to a high purity for subsequent crystallographic analysis.

## Conclusion

Our findings support the *in silico* predictions of the essentiality of GapN and indicate that the enzyme might be a suitable target for new antimicrobial compounds.

### 386-MPP

The 5'-nucleotidase S5nA is dispensable for evasion of phagocytosis and biofilm formation in ***Streptococcus pyogenes***

M. L. Dangel<sup>1</sup>, \*E. Zitzow<sup>1</sup>, J. C. Dettmann<sup>1</sup>, M. Schakat<sup>1</sup>, M. Krogull<sup>1</sup>, B. Kreikemeyer<sup>1</sup>, T. Fiedler<sup>1</sup>

<sup>1</sup>Universitätsmedizin Rostock, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Rostock, Germany

## Question

5'-nucleotidases (5'NT) are widespread among all domains of life. The enzymes hydrolyze phosphate residues from nucleotides and nucleotide derivatives. In some pathobiontic bacteria, 5'NT contribute to immune evasion by dephosphorylating adenosine mono-, di-, or tri-phosphates, thereby either decreasing the concentration of pro-inflammatory ATP or increasing the concentration of anti-inflammatory adenosine, both acting on purinergic receptors of phagocytic cells. The strict human pathogen *Streptococcus pyogenes* expresses a surface-associated 5'-NT (S5nA) under infection conditions that has previously been discussed as a potential virulence factor. Here we investigated the impact of S5nA gene deletions on virulence traits of *S. pyogenes*.

## Methods

The S5nA genes were deleted in three *S. pyogenes* strains by homologous recombination. Complement strains expressed S5nA from a pAT19-based plasmid. Surface 5'NT activity was determined by measuring the release of phosphate in a colorimetric assay. Survival in human blood or plasma and phagocytosis were investigated by assessing viable counts of the bacteria before and after (i) 3 h incubation in blood/plasma or (ii) 30 min incubation with

freshly isolated neutrophils. Larvae of *Galleria mellonella* were infected with the bacteria and survival of the larvae was observed for 7 d.

## Results

Our data show that deletion of the S5nA gene does not significantly affect growth in human blood, evasion of phagocytosis by neutrophils, formation of biofilms and virulence in an infection model with larvae of the greater wax moth *Galleria mellonella* in *S. pyogenes* serotypes M6, M18 and M49.

## Conclusions

The surface-associated 5'-nucleotidase S5nA is dispensable for evasion of phagocytosis and biofilm formation in *S. pyogenes*.

### 387-MPP

The external pH regulates the activity of the ***Yersinia enterocolitica*** type III secretion system

\*S. Wimmi<sup>1</sup>, A. Balinovic<sup>2</sup>, H. Jeckel<sup>3</sup>, D. Linke<sup>4</sup>, K. Drescher<sup>3</sup>, U. Endesfelder<sup>2</sup>, A. Diepold<sup>1</sup>

<sup>1</sup>Max-Planck-Institut für terrestrische Mikrobiologie, Ecophysiology, Marburg, Germany

<sup>2</sup>Max-Planck-Institut für terrestrische Mikrobiologie, Department of Systems and Synthetic Microbiology, Marburg, Germany

<sup>3</sup>Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany

<sup>4</sup>University of Oslo, Institute for Biosciences, Oslo, Norway

Introduction: *Yersinia enterocolitica* is a gram negative, gastrointestinal, human pathogen. One of its major virulence factors is the type III secretion system (T3SS). It resembles a molecular syringe, commonly known as injectisome, which establishes a direct cytosolic connection between the bacterial and host cytoplasm. While the structure of the injectisome is well-defined and can be separated in static and mobile components, little is known about the molecular function and regulation of T3SS activity.

Question and methods: We aimed to characterize the molecular regulation of T3SS activity and its physiological role during infection. To this aim, we combined live cell fluorescence microscopy, single particle tracking, bioinformatic analysis and functional assays. We discovered that the external pH, which strongly varies during *Y. enterocolitica*'s passage through the gastrointestinal tract, has a strong impact on the mobile cytosolic components of the T3SS.

Results: Our results show that at low external pH, as encountered by the bacteria during the passage of the gastrointestinal tract, the cytosolic components of the injectisome are temporarily released, thereby preventing effector secretion. Low external pH is sensed in the periplasm by the inner membrane component SctD, which partially dissociates at low external pH, and transmits the signal to the cytosolic T3SS components, which unbind from the injectisome. Once the neutral pH is restored, this effect is reversed, allowing reassociation of the cytosolic components, which results in a fast activation of the injectisome at the Peyer's patches.

Conclusion: Our findings indicate the presence of an adaptive regulatory interface between the membrane-bound and cytosolic T3SS components. This mechanism regulates T3SS activity in response to environmental conditions and provides an additional layer of regulation. This allows to limit

the otherwise unspecific secretion of the injectisome to favorable environmental conditions, thereby preventing the loss of valuable effectors or immune responses, and ultimately promoting successful infections. (Wimmi et al., manuscript in preparation)

### 388-MPP

Aggregative Adherence Fimbriae Type I Determine the Superior Adherence Phenotype of Enterohemorrhagic *Escherichia coli* O104:H4

\*M. Berger<sup>1</sup>, U. Dobrindt<sup>1</sup>, P. Berger<sup>1</sup>, P. Dersch<sup>2</sup>, P. Schiller<sup>1</sup>, M. Knödler<sup>1</sup>

<sup>1</sup>UKM, Institut für Hygiene, Münster, Germany

<sup>2</sup>WWU, ZMBE, Münster, Germany

**Introduction:** The exceptionally virulent German enterohemorrhagic *Escherichia coli* (EHEC) O104:H4 outbreak strain did not only encode a *stx2* phage, but also aggregative adherence fimbriae (AAF), the characteristic virulence marker of enteroaggregative *Escherichia coli* (EAEC). We have recently shown that significantly more EHEC O104:H4 bacteria (encoding AAF/I) were attached to epithelial cells than bacteria of one of the closest known relatives (EAEC 55989, encoding AAF/III). The tight adherence of high numbers of EHEC O104:H4 cells was proposed to have significantly contributed to the exceptional pathogenicity of the strain, but it was not clear if this phenotype could be directly attributed to AAF/I.

**Objectives:** Our objective was the phenotypic characterization of AAF/III with respect to AAF/I by heterologous expression in EAEC 55989 *agg3*-.

**Materials and methods:** We heterologously expressed AAF/I, III and the control AAF operons IV and V in EAEC 55989 *agg3*-. We analyzed the bacterial surface by electron microscopy, measured sedimentation speed following autoaggregation, analyzed mixed aggregates by fluorescence microscopy, measured biofilm formation and microscopically analyzed the AAF mediated adhesion to cultured epithelial cells.

**Results:** We showed the presence of AAF/I, III, IV and V on the bacterial surface, and that heterologous expression of all AAF led to rapid sedimentation of the aggregates, albeit at different speed. We demonstrated that more or less mixed aggregates are formed, if bacteria expressing different AAF were mixed. Biofilm formation capacity was overall anti-correlated with sedimentation speed following autoaggregation. EAEC 55989 *agg3*- expressing AAF/I were forming larger aggregates on HEp2-cells than the same strain expressing AAF/III, IV and V.

**Conclusion:** The previously observed exceptionally high numbers of EHEC O104:H4 on cultured epithelial cells are directly dependent on AAF/I fimbria expression.

### 389-MPP

Characterisation of *Streptococcus suis* mutants in PCLS and ALI cultures

\*M. Dresen<sup>1</sup>, D. Vötsch<sup>1</sup>, Y. B. Weldearegay<sup>1</sup>, M. Willenborg<sup>1</sup>, M. Rohde<sup>2</sup>, J. Arenas Busto<sup>3</sup>, A. de Greeff<sup>3</sup>, A. Nerlich<sup>1</sup>, P. Valentin-Weigand<sup>1</sup>

<sup>1</sup>University of Veterinary Medicine Hannover, Foundation, Institute for Microbiology, Hannover, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Central Facility for Microscopy, Braunschweig, Germany

<sup>3</sup>Wageningen BioVeterinary Research, Department of Infection Biology, Lelystad, Netherlands

*Streptococcus suis*, an emerging zoonotic pathogen, can cause invasive disease in pigs, primarily weaning piglets, with symptoms such as acute sepsis, meningitis, endocarditis, pneumonia and arthritis. Importantly, similar pathologies are also reported in humans. In order to gain a better understanding of the pathogenesis of *S. suis* infection, we characterized the role of new candidate virulence factors in *S. suis* mutants. The mutants have been generated on the basis of an in vivo screening of a transposon library.

As the natural infection of pigs with *S. suis* occurs via the respiratory tract, we studied the interaction of the mutants with cells of the respiratory tract. For this, we applied precision-cut lung slices (PCLS) and air-liquid interface (ALI) cultures with tracheal and bronchial epithelial cells. Both models mimic very closely the porcine respiratory epithelium. The *ex vivo* PCLS model preserves the structural and functional integrity of the lung, including the ciliary activity. In the ALI system, the well-differentiated respiratory epithelial cells build a pseudostratified epithelium, containing ciliated and mucin-producing cells as well as tight junctions. We investigated the adherence, colonization and invasion capacity of *S. suis* mutants in the above described cell culture models. In addition, microbial effects on bronchial epithelial cells, e.g., cytotoxicity, and effects on ciliary motility as well as bronchoconstriction, were analyzed. Altogether we tested 24 different mutants in the PCLS model. There were no effects on the ciliary activity detectable for up to 24 hpi. Moreover, no differences in cytotoxicity could be observed. One mutant showed a lower bacterial growth in the supernatant and a lower adherence to PCLS compared to the wildtype. The low adherence was also confirmed via immunofluorescence analysis. Besides, immunofluorescence analysis revealed morphological growth defects of a second mutant, which shows an irregular cell shape and size. Furthermore, mispositioned division septa were observed by electron microscopy. In the future such virulence determinants can be used as targets for vaccines or "pathotyping" diagnostic.

### 390-MPP

An enigmatic non-coding RNA links *Staphylococcus aureus* physiology to antibiotic resistance

\*E. S. Ibrahim<sup>1</sup>, E. M. El-Hossary<sup>2</sup>, U. Holzgrabe<sup>3</sup>, K. Ohlsen<sup>1</sup>

<sup>1</sup>Institute for Molecular Infection Biology, Würzburg, Germany

<sup>2</sup>National Centre for Radiation Research & Technology, Egyptian Atomic Energy Authority, Cairo, Egypt

<sup>3</sup>Institute of Pharmacy and Food Chemistry, Würzburg, Germany

Although non-coding RNAs (ncRNAs) are vital regulatory elements that coordinate shaping the Gram-positive human pathogen *Staphylococcus aureus* phenotype, they remain understudied. In this study, we explore the role of an ncRNA in mediating the oxidative stress response and conferring phenotypic resistance against a novel potential antibiotic. Using RNA-seq, we compared the transcriptome in low to high putative oxidative stress using the bisquaternary bisnaphthalimide compound MT02 (a novel antistaphylococcal agent). Under normal conditions, low-level transcription of a *Firmicutes*-conserved oxidoreductase enzyme (SAUSA300\_RS04635) is associated with undetectable levels of an antisense ncRNA. Under putative oxidative stress induced by MT02, both the ncRNA and SAUSA300\_RS04635 are massively upregulated to mediate the stress response. Sequence analysis of the genomic locus shows two potential promoters for SAUSA300\_RS04635 leading to two corresponding mRNAs with varying 5'UTRs. Reporter assay for the activities of the mRNA primary promoter and the ncRNA promoter (P<sub>SAUSA300\_RS04635</sub> and P<sub>ncRNA</sub>) confirms that both transcriptions start from promoters characterized by two sets



of inverted repeats and climax by the mid log-phase. *PSAUSA300\_RS04635* and *P<sub>ncRNA</sub>* have similar transcriptional activities. We are developing a reporter assay to elucidate the possible interaction between the messenger and non-coding RNA. The interaction may influence the oxidoreductase level in the bacterium leading to the MT02 resistant phenotype and, moreover, the oxidative stress mediation. All together, we highlight unexpected transcriptional flexibility of *S. aureus* by unleashing the ncRNA thought to regulate a conserved metabolic function. Investigating the ncRNA will help for better understanding of the regulation networks in staphylococci.

### 391-MPP

The role of the *fdeC* gene of avian pathogenic *Escherichia coli* (APEC) strain IMT5155 in adhesion to chicken intestinal epithelial cells

\*A. Aleksandrowicz<sup>1,2</sup>, R. Kolenda<sup>1</sup>

<sup>1</sup>Wrocław University of Environmental and Life Sciences, Department of Biochemistry and Molecular Biology, Wrocław, Poland

<sup>2</sup>University of Wrocław, Department of Microbiology, Wrocław, Poland

## 1. Introduction

*E. coli* typically colonizes human and animal gastrointestinal tract as commensal. Pathogenic *E. coli* utilize acquired virulence factors to colonize host tissues during infection. APEC is cause of poultry colibacteriosis that generates extensive losses in poultry industry worldwide. Successful disease development of APEC depends on the initial step—adhesion to host cells. A number of fimbrial and non-fimbrial adhesins play a significant role in this interaction. Our results from transposon library screen identified gene *fdeC* as a putative adhesion factor.

## 2. Objectives

So far the knowledge about FdeC contribution to APEC host colonization is limited. Therefore our main objective was to determinate the role of the *fdeC* gene in APEC adhesion to chicken intestinal epithelial cells.

## 3. Material and methods

To create a deletion mutant of the *fdeC* gene, Datsenko-Wanner protocol was used. Mutant strains were tested for changes in morphology and growth speed. To examine the *fdeC* contribution to adhesion ability, adhesion assays with CHIC-8E11 cell line were performed. All strains used for assays were grown: overnight in LB (37°C, 16h; 180 rpm) or overnight cultures were diluted and grown to OD<sub>600</sub> = 0.5, 1 or 2 (37°C or 42°C; 220 rpm). Bacterial suspensions were diluted and added to the cell line with a MOI=100. The bacteria were allowed to adhere to the monolayer for 3h, at 37°C or 42° and counted using spot test onto agar plates

## 4. Results

Tested strains did not change their morphology. Speed of growth in LB medium was similar for all bacteria. However, in infection medium, a growth rate difference between strains was visible after 3h of incubation. Wild type and mutants strains adhered similarly to monolayer at 37°C. A slight increase in adhesion was observed for *fdeC* deletion mutant grown to OD<sub>600</sub>=1 or 2 at 37°C. At 42°C, the same mutant grown to OD<sub>600</sub>=1 showed analogous result.

## 5. Conclusion

The preliminary role of the FdeC protein in adhesion to chicken intestinal epithelial cells has been determined. However, to fully understand this mechanism, further experiments should be conducted.

### 392-MPP

The effect of cis-2-decenoic acid on biofilm formation of

#### *Clostridioides difficile*

\*J. Fischer<sup>1</sup>, H. Bahl<sup>1</sup>

<sup>1</sup>Universität Rostock/ Institut für Biowissenschaften, Mikrobiologie, Rostock, Germany

Biofilm formation on implants cause severe infections which can lead to the failure of the implant. The therapy of biofilm related infections is difficult. Antibiotic treatments are less effective and need high concentrations, resulting also in a dysbiosis of the gut microbiota. This leads to outgrowth of *Clostridioides difficile* spores with consecutive antibiotic-associated diarrhea and irritable bowle diseases like pseudomembranous colitis. These inflammations cause the so called leaky gut whereas *C. difficile* then is able to enter the blood stream and colonizes abiotic surfaces, eg. implant materials. The treatment of the consecutive biofilm related infection with antibiotics induces a vicious circle. Thus, alternative therapeutic agents are needed. Cis-2-decenoic acid (C2DA) is a fatty acid produced by *Pseudomonas aeruginosa* which can disperse microbial biofilms. The aim of this study was to analyze biofilm formation by *C. difficile* and to determine the effect of C2DA on the adhesion to surfaces *in vitro*. Overnight grown cultures of *C. difficile* 630 and VPI 10463 strains were transferred to 24-well plates and cultured up to 72 h. The biofilm formation was quantified by crystal violet staining and visualized by fluorescence microscopy after live-dead staining. Both strains exhibited similar biofilm formation after 24 h. A medium change every 24 h led to a stable and increased biofilm formation up to 72 h. To determine the effect of C2DA, different concentrations were added into the medium. A concentration of 125 µg/µl was able to impair biofilm formation. We conclude that under the conditions tested, C2DA is a potential candidate for treating biofilm related infections by *C. difficile*.

### 393-MPP

#### Analysing the fibronectin binding properties of *Bartonella henselae* adhesin A

\*A. Thibau<sup>1</sup>, D. J. Vaca<sup>1</sup>, D. Linke<sup>2</sup>, V. A. J. Kempf<sup>1</sup>

<sup>1</sup>University Hospital, Goethe University Frankfurt, Institute for Medical Microbiology and Infection Control, Frankfurt am Main, Germany

<sup>2</sup>University of Oslo, Department of Biosciences, Section for Genetics and Evolutionary Biology, Oslo, Norway

**Introduction:** *Bartonella henselae* infections can result in cat scratch disease, endocarditis and vasculoproliferative disorders (e.g. bacillary angiomatosis). *Bartonella* adhesin A (BadA) is a trimeric autotransporter adhesin (TAA) and mediates bacterial adhesion to human endothelial host cells (EC) or extracellular matrix (ECM) proteins (e.g. fibronectin). Fibronectin is a high molecular weight glycoprotein mediating the initial bacterial adhesion to host cells presumably via bridging to α5β1-integrins.

**Objectives:** To identify possible binding site(s) of BadA to fibronectin and to model and produce peptides that inhibit bacterial adhesion to ECs and ECM proteins. Findings might be used for developing bacterial "anti-ligands" as a new

class of antibiotics. For this, an exact domain analysis of BadA between various *Bartonella* spp. is decisive.

**Materials & Methods:** Identification of potential fibronectin binding site(s) is performed via the construction of truncated and modified BadA fusion proteins. Affinity assays are carried out via ELISA. BadA-gene sequences are analysed using long-read PacBio SMRT sequencing and BadA-expression is verified via electron microscopy. Functional read outs will be done using various *ex vivo* and *in vitro* infection models and by using lab-on-chip devices (nanobiosensors).

**Results & Conclusion:** Previous research demonstrated the crucial role of fibronectin in the first attachment of *B. henselae* to human ECs and ECM proteins. ELISA fibronectin affinity assays using truncated BadA fusion proteins have shown the significance of the BadA length and the importance of certain domain(s) within BadA. Furthermore, anti-BadA antibodies were developed and a clean BadA-negative *B. henselae* mutant is being constructed via Gibson cloning. Additionally, the genomic BadA sequences of various *Bartonella* spp. are determined via state-of-the-art long-read PacBio SMRT Sequencing. BadA, derived from *B. henselae* Marseille, has even a higher molecular weight (3.972 amino acids) than previously demonstrated (3.082 amino acids). These results represent a stable basis for the further functional approaches to verify fibronectin-binding BadA-domains.

### 394-MPP

Simulation of blood flow-mediated shear stress in pneumococcal cell culture infection

H. Jagau<sup>1,2</sup>, K. Lahme<sup>1</sup>, I. K. Behrens<sup>1,3</sup>, M. Rohde<sup>4</sup>, M. Steinert<sup>1,5</sup>, \*S. Bergmann<sup>1</sup>

<sup>1</sup>Technische Universität Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany

<sup>2</sup>Lund University, Department of Clinical Sciences, Lund, Sweden

<sup>3</sup>Ludwigs-Maximilians-Universität München, Max von Pettenkofer Institut, Chair of Medical Microbiology and Hospital Epidemiology, München, Germany

<sup>4</sup>Helmholtz Center for Infection Research, Central Facility for Microscopy, Braunschweig, Germany

<sup>5</sup>Helmholtz Center for Infection Research, Department of Infection Biology, Braunschweig, Germany

### Introduction

*Streptococcus pneumoniae* (pneumococcus) can cause severe systemic infections (1). Recently, we identified the surface-associated enolase of *S. pneumoniae* as binding factor for globular and multimerized Von Willebrand Factor (VWF), which is secreted by Weibel Palade bodies of vascular endothelial cells (2). The mechano-sensitive VWF responds to shear stress with conformational changes mediating VWF multimerization to long protein strings (3).

### Objectives

Functional analyses of the interaction between pneumococci and VWF on endothelial cell surfaces requires specific physiological parameters of the blood flow. Here, we present a cell culture infection model using a microfluidic pump system. This system enables the simulation of shear stress levels, which are typically generated by the blood flow in the vascular system.

### Materials and Methods

A microfluidic cell culture infection model was established using the pump system from the company ibidi® in combination with microscopic real time imaging and differential immunofluorescent staining.

### Results

We first optimized the attachment of primary human endothelial cells to the microslide surface and created a two-step flow procedure to promote a gentle shear force adaptation. After cell differentiation in flow, multimerized VWF strings were generated in response to the shear stress and attachment of pneumococci to VWF strings in flow was analysed by fluorescence microscopy in real time (4). In addition, after probe fixation and differential immunofluorescence staining, bacterial adhesion was quantified and evaluated by confocal laser scanning microscopy.

### Conclusion

This microfluidic cell culture infection model is suitable for visualization and quantitative evaluation of pneumococcal adhesion to vascular cell surfaces in flow and can be easily adapted to study interactions of other bacterial or viral pathogens and even parasites with the host endothelium.

### References

- (1) Cartwright, 2002, doi: 10.1007/s00431-001-0907-3
- (2) Jagau, et al., 2019a, doi: 10.3389/fmicb.2019.00511
- (3) Valentijn and Eikenboom, 2013, doi: 10.1111/jth.12160
- (4) Jagau, et al., 2019b, doi: 10.3791/60323

### 395-MPP

Profiling pathogenicity mechanisms of *Bordetella pertussis* & *B. parapertussis*, the causative agents of whooping cough, a re-emerging disease, by proteomics approaches

\*K. Surmann<sup>1</sup>, Y. Lamberti<sup>2</sup>, J. Alvarez Hayes<sup>2</sup>, J. H. Cafiero<sup>2</sup>, J. M. Oviedo<sup>2</sup>, J. Gorgojo<sup>2</sup>, H. Valdez<sup>2</sup>, B. Vecerek<sup>3</sup>, J. Holubova<sup>3</sup>, M. Depke<sup>1</sup>, V. M. Dhople<sup>1</sup>, F. Schmidt<sup>1</sup>, M. E. Rodriguez<sup>2</sup>, U. Völker<sup>1</sup>

<sup>1</sup>University Medicine Greifswald, Functional Genomics, Greifswald, Germany

<sup>2</sup>CINDEFI, Fac. de Cs. Exactas-UNLP, La Plata, Argentina

<sup>3</sup>Institute of Microbiology of the ASCR, v.v.i., Prague, Czech Republic

### Question

Even in fully vaccinating countries, whooping cough caused by *Bordetella pertussis* (*Bp*) and *B. parapertussis* (*Bpp*), is regarded as re-emerging disease. Besides vaccine inefficiency, intracellular survival of the pathogens leads to dissemination in the population.

In order to elucidate bacterial adaptation to infection related conditions, we have analysed the global proteome of both pathogens to iron-limitation and additionally, upon internalization of *Bp*.

### Methods

*Bp* Tohama I, an isogenic  $\Delta hfq$  mutant, and *Bpp* CN2591 were cultivated in iron-replete and iron-depleted Stainer-

Scholte medium until late exponential growth phase. Bacterial proteomes were analysed by nanoLC-MS/MS. In addition, human monocytic THP-1 cells were infected with *Bp* Tohama I (MOI 150) and after 2 h, non-internalized bacteria were killed with polymyxin B sulfate. The proteome of recovered intracellular *Bp* was examined 3 h and 48 h post infection and compared to extracellular *Bp* grown under similar conditions. In addition, infection assays were repeated with mutants in *BP0414* or *hfq*, encoding an MgtC homologue or the RNA-chaperone Hfq, respectively, to assess their impact onto intracellular survival by bacterial counting.

## Results

Hfq modulates 33% of *Bp* proteins regulated during iron limitation (Alvarez Hayes *et al.*, *J Proteomics*, 2019) and influenced bacterial survival. Upon internalization, 40% of the detected proteins showed altered levels including proteins involved in stress response, iron uptake, metabolism, and virulence (Lamberti *et al.*, *J Proteomics*, 2016). A follow-up study revealed that MgtC plays a role in the adaptation of *Bp* to the acidic conditions inside phagosomes (Cafiero *et al.*, *PLoS One*, 2018). Moreover, we identified proteins of stress resistance and virulence exclusively in *Bpp* that might help explaining differences in pathogenesis of both species (Oviedo *et al.*, *J Proteomics*, 2019).

## Conclusions

Here, we present comprehensive proteome data of the pertussis causing agents that help to better understand their pathogenicity mechanisms and pave the way for follow-up studies of the infected host.

### 396-MPP

#### Antimicrobial efficiency of antisense PNAs in *Streptococcus pneumoniae*

\*C. Abt<sup>1</sup>, G. Barkowsky<sup>1</sup>, A. L. Lemster<sup>1</sup>, R. Pappesch<sup>1</sup>, S. Krüger<sup>1</sup>, A. Jacob<sup>2,3</sup>, B. Kreikemeyer<sup>1</sup>, N. Patenge<sup>1</sup>

<sup>1</sup>Institut für Medical Microbiology, Virology and Hygiene, Rostock, Germany

<sup>2</sup>Functional Genome Analysis, Deutsches Krebsforschungszentrum, Heidelberg, Germany

<sup>3</sup>Peps4LS, Heidelberg, Germany

## Introduction

*Streptococcus pneumoniae* is the most common bacterial cause of pneumoniae and responsible for multiple other infectious syndromes as meningitis and otitis media in children. Resistance to penicillins, macrolides and fluoroquinolones are increasing. Since introduction of pneumococcal conjugate vaccine (PCV), vaccinal serotypes are replaced by non-vaccine serotypes. Therefore, novel therapies are needed. Antisense peptide nucleic acids (PNAs) were shown to reduce growth of several pathogenic bacteria, but spontaneous cellular PNA uptake is restricted.

## Objectives

PNA can be coupled to cell-penetrating peptides (CPPs) that support PNA translocation. Here, we investigate the effect of three CPP-coupled anti-*gyrA* PNAs and anti-*rpoB* PNAs on *S. pneumoniae* growth.

## Materials and methods

Three different *S. pneumoniae* strains (TIGR4, D39, 19F) were investigated in this study: 10<sup>5</sup> CFU/ml bacteria were incubated with 2 – 20 µM CPP-coupled anti-*gyrA* and anti-*rpoB* PNAs for 6 h at 37 °C. Reduction of bacterial counts was determined in comparison to the untreated sample.

## Results

(RXR)<sub>4</sub>XB peptide-coupled antisense PNAs reduced bacterial counts *in vitro*. PNAs linked to HIV-1 TAT caused less reduction, while peptide oligolysine (K8) had no effect. Antimicrobial efficiency of the constructs was serotype specific. Furthermore, the antimicrobial activity depended on the target gene: anti-*rpoB* caused stronger cell count reduction than anti-*gyrA* PNAs.

## Conclusion

This work provides a proof of principle that CPP-antisense PNAs can act bactericidal on *S. pneumoniae*. (RXR)<sub>4</sub>XB peptide-coupled anti-*gyrA* and anti-*rpoB* PNAs caused significant cell count reduction of the pathogen. Further CPPs should be tested to mediate translocation of PNAs into most prevalent serotypes of *S. pneumoniae*.

### 397-MPP

#### *Streptococcus pneumoniae* differently affects primary microglia and bone marrow-derived macrophages

\*F. Jennert<sup>1</sup>, J. Seele<sup>2,3</sup>, R. Nau<sup>2,3</sup>, T. P. Kohler<sup>4</sup>, S. Hammerschmidt<sup>4</sup>, P. Valentin-Weigand<sup>1</sup>

<sup>1</sup>University of Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany

<sup>2</sup>University Medical Center Göttingen, Institute for Neuropathology, Göttingen, Germany

<sup>3</sup>Evangelisches Krankenhaus Göttingen-Weende, Department of Geriatrics, Göttingen, Germany

<sup>4</sup>Greifswald University, Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Germany

*Streptococcus (S.) pneumoniae* (pneumococcus) is a commensal bacterium of the human upper respiratory tract. It is the most common cause of bacterial meningitis and meningoencephalitis in humans with a high mortality rate of 20-30%. Among the major pneumococcal virulence factors are the capsular polysaccharide (CPS), the cytolysin pneumolysin (Ply) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which is mainly produced by pyruvate oxidase (SpxB). The role of these virulence factors in the pathogenesis of meningitis is still not fully understood. In the central nervous system (CNS), glial cells are considered to play an important role during inflammation and injury of the brain in bacterial meningitis. Microglia are the resident macrophages of the CNS. To characterize the response of these specific brain phagocytes to pneumococcal infection, a comparative analysis of primary microglia cells and bone marrow-derived macrophages (BMDM) was performed.

Mixed glial cell cultures were prepared from neonatal C57BL/6 mice and microglial cells were isolated. Bone marrow-derived cells were prepared from adult C57BL/6 mice and differentiated into BMDM. Microglia and BMDM were infected with wild-type *S. pneumoniae* D39 as well as with mutants deficient for CPS, Ply, or SpxB. The survival of phagocytes during the infection was visualized by microscopy using Hoechst and propidium iodide staining, bacterial growth was determined by plating. Furthermore, both primary phagocyte cultures were exposed to different concentrations of recombinant Ply or H<sub>2</sub>O<sub>2</sub>, and survival was measured.

Primary microglia were killed during pneumococcal infection independently of Ply, whereas BMDM were not affected. Catalase protected microglia during pneumococcal infection. Treatment of cells with Ply led to a dose-dependent cytotoxic effect in both phagocyte types. However, very high concentrations of Ply were necessary for this effect.

In conclusion, primary microglia and BMDM react in a different manner to pneumococcal infection, Ply and H<sub>2</sub>O<sub>2</sub>.

---

### 398-MPP

Transcriptomic Response of Human Pharyngeal Epithelial Cells to (Co-)infection with *Streptococcus pyogenes* and Influenza A Virus

\*K. Strey<sup>1</sup>, S. Sueto<sup>2</sup>, R. Saß<sup>1</sup>, S. Kurrer<sup>1</sup>, N. Patenge<sup>1</sup>, W. I. Barrantes Bustinza<sup>2</sup>, B. Kreikemeyer<sup>1</sup>

<sup>1</sup>Institute of Medical Microbiology, Virology and Hygiene, Rostock, Germany

<sup>2</sup>Institute for Biostatistics and Informatics in Medicine and Ageing Research, Rostock, Germany

The human pathogen *Streptococcus pyogenes* causes a variety of mild to serious diseases. Influenza A viruses (IAVs), are enveloped (-)ssRNA viruses and cause seasonal outbreaks of respiratory disease (influenza). Annual influenza vaccination is recommended to prevent disease onset, but concern regarding a viral strain with pandemic potential remains. The course of disease is exacerbated in case of viral-streptococcal co-infections.

The aim of the study is to identify pathogen-specific host cell responses following mono and co-infections.

The present project employed a cell culture-based model to investigate mono-infection of the pharyngeal epithelial cell line Detroit 562 with *S. pyogenes* serotype M1 strain AP1 (M1), *S. pyogenes* serotype M49 strain 591 (M49) or IAV (H1N1). Host viability was assessed via flow cytometry. Furthermore, bacterial adherence to and viral propagation within host cells was determined. Following infection, ELISAs were performed to evaluate cytokine secretion and cell death-related marker expression/ abundance was examined by qPCR and western blot. Additionally, host cell total RNA was collected for subsequent transcriptome analyses.

M49 adherence to Detroit 562 was increased. The number of viable host cells was significantly reduced upon infection with M49 compared to M1. M49 infection resulted in increased *cas3* gene expression. This effect was not observed following infection with neither M1 nor IAV. Infection with each of the three pathogens caused decreased IL-8 secretion. Transcriptome analyses revealed different response pathways following IAV and streptococcal infection. Moreover, transcriptomic changes upon M1 and M49 infection differed remarkably, with M1 inducing expression of genes involved in the electron transport chain.

In the pharyngeal epithelial cell infection model a pathogen-specific impact on the host cell response could be demonstrated. M49 compromised host cell viability. Under all three conditions, decreased IL-8 concentration in the supernatant was observed. Transcriptome data show that following mono-infection with each of the three pathogens different host cell pathways were influenced.

---

### 399-MPP

The author has not agreed to a publication.

### 400-MPP

LITESEC-T3SS - Light-controlled protein delivery into eukaryotic cells with high spatial and temporal resolution

\*F. Lindner<sup>1</sup>

<sup>1</sup>Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany

#### 1. Introduction

The type III secretion system (T3SS) is a needle-like structure that is used by many pathogenic Gram-negative bacteria to translocate effector proteins from the bacterial cytosol into host cells. It has also been successfully used to deliver non-native cargo into various host cells for different purposes such as vaccination or immunotherapy. However, bacteria are not restricted to specific target cells, and T3SS inject effector proteins into any eukaryotic host cells upon contact. Lack of target specificity is therefore a main obstacle in the further development and application of the T3SS as a specific protein delivery tool.

#### 2. Objectives

Our lab has shown that the cytosolic complex of the T3SS acts as a highly dynamic interface, in which parts permanently exchange and shuttle between the cytosol and the T3SS, and that this exchange behaviour is linked to protein secretion. These findings allow for a completely new way to control the T3SS activity via specific sequestration and release of cytosolic T3SS components. By combining light-controllable optogenetic interaction switches with essential T3SS components, we aimed to control T3SS-based protein translocation with spatial and temporal precision.

#### 3. Materials & Methods

We used bacterial secretion assays, host-cell infection assays and fluorescence microscopy to monitor light-controlled protein secretion and translocation by the LITESEC-T3SS.

#### 4. Results

We successfully established optogenetic interaction switches in bacteria and combined them with the cytosolic T3SS component SctQ, to create the LITESEC-T3SS system. We could show that the secretion of native effector proteins or non-native cargo proteins, as well as the translocation of these proteins into eukaryotic host cells can be efficiently controlled by light in the engineered strains.

#### 5. Conclusion

The combination of optogenetic interaction switches with a dynamic essential cytosolic component of the T3SS enables reversible spatial and temporal control of the T3SS function. This enhances the usage of the T3SS as a specific protein delivery tool into eukaryotic cells and enables widespread applications. Ref: Lindner et al.(2019) bioRxiv 807461

---

### 401-MPP

Development of a multi-cell *in vitro* model of the meningeal blood-cerebrospinal fluid barrier to study *Neisseria meningitidis* infection

\*L. Endres<sup>1</sup>, B. Kim<sup>1</sup>, M. Christodoulides<sup>2</sup>, A. Schubert-Unkmeir<sup>1</sup>

**Introduction:** *Neisseria meningitidis* (*Nm*, meningococcus) is a human-specific pathogen that can gain access to the central nervous system (CNS) by crossing the meningeal blood-cerebrospinal fluid barrier (mBCSFB). Much research has been conducted examining the endothelial cells of the mBCSFB, but little is known about bacterial interactions with other cell types that comprise the barrier such as leptomeningeal cells (LMCs). Including such cells in the study of *Nm* interaction at the mBCSFB has the potential to provide novel insights into the mechanisms underlying bacterial meningitis.

**Objectives:** Here we sought to develop a multi-cell mBCSFB model that is entirely human based to examine *Nm* interaction *in vitro*.

**Methods:** Human brain endothelial cells (BECs) (hCMEC/D3) and LMCs derived from tumor biopsies are being used in development of the model. Gentamycin protection assays were conducted to estimate meningococcal adherence and invasion. Immunofluorescence of the tight junction components was conducted to investigate impacts of bacterial challenge on barrier integrity. qPCR was used to determine the cellular response to infection. Finally, confocal and super-resolution microscopy are being explored as tools to visualize localization of meningococci in the human mBCSFB *in vitro* model.

**Results:** We observed modest amounts of meningococcal adherence to the leptomeningeal cells, whilst detecting very little bacterial invasion. Aside from expression of characteristic markers of the LMCs, we observed expression and junctional localization of tight junction proteins ZO-1 and Occludin. Preliminary results from co-culture experiments with BECs suggest barrier tightening upon addition of the LMCs as measured by trans-endothelial electrical resistance.

**Conclusion:** Our work highlights the usefulness of LMCs for the study of *Nm* interaction at the mBCSFB. Future aims include elucidating the effects of infection on expression and localization of the tight junction proteins. Co-culture with BECs will lead to an advanced co-culture *in vitro* model of the human mBCSFB, enabling study of meningococcal interaction that more accurately mimics the meningeal microenvironment.

---

#### 402-MPP

##### **Augmented translocation of *S. aureus* across the N-acetylcysteine (ACC) treated lung epithelial barrier in vitro**

\*M. Fröhlich<sup>1</sup>, M. Brück<sup>1</sup>, C. Musholt<sup>1</sup>, B. Shuhen<sup>1</sup>, B. Schulte<sup>1</sup>, M. Herrmann<sup>1</sup>, S. Niemann<sup>1</sup>

<sup>1</sup>UKM Münster, Institute of Medical Microbiology, Münster, Germany

**Introduction:** Upon respiratory infections with *Staphylococcus aureus*, lung epithelia produce mucus as a protective mechanism against bacterial invasion. N-acetylcysteine (ACC) is a mucolytic and reducing agent that is used as a cough medicine. In vitro studies of respiratory infections indicate that ACC improves the antibacterial properties of antibiotics. Furthermore, data suggest that it has the ability to interfere with biofilm formation and to disrupt biofilms. However, high susceptibility of cells against bacterial infection when treated with ACC could be reported.

**Objectives:** The aim of this study was to examine the mucolytic capacity of ACC on lung epithelial cells in order to improve cellular invasion of *S. aureus*.

**Material and methods:** Lung epithelial cells A549 and Calu-3 were cultured on Transwell inserts. Cells were pre-treated with ACC before infection with the *S. aureus* laboratory strain 6850 at a multiplicity of infection of one or five. As controls, untreated cells or cells that were treated only with bacteria were used. Three and 24 hours post-infection (hpi), media from the apical, baso-lateral, and the cellular compartment were collected and plated on blood agar plates. In addition, the activation of host cells and cytotoxicity were assessed.

**Results:** Pre-treatment of both cell types with ACC resulted in an increased translocation of *S. aureus* through the cellular barrier, as compared to control cells without ACC. With increasing time of pre-treatment, bacterial translocation was enhanced at 3 hpi. At 24 hpi, we could observe even higher translocation for all treatments. In A549, we could detect increased cell death when cells were treated with ACC alone and in combination with *S. aureus*. Furthermore, enhanced gene expression of ICAM-1, TNF $\alpha$ , and IL-1 $\beta$  could be detected in A549 already at 3 hpi.

**Conclusion:** Our data suggest that treatment of lung epithelial cells in vitro with ACC might enhance the inflammatory response towards bacterial infection and results in increased apoptosis. Therefore, the bacteria could easier breach the cellular barrier. Whether similar effects would arise in vivo too certainly needs to be investigated.

---

#### 403-MPP

##### **Characterization of natural products as novel inhibitors of the caseinolytic protease P**

\*A. Illigmann<sup>1</sup>, F. Wolf<sup>2</sup>, L. Kaysse<sup>2</sup>, H. Brötz-Oesterhelt<sup>1</sup>

<sup>1</sup>University of Tübingen, Microbial Bioactive Compounds, Tübingen, Germany

<sup>2</sup>University of Tübingen, Pharmaceutical Biology, Tübingen, Germany

Fighting antibiotic resistance is a challenging task. Therefore, innovative antibacterial agents urgently need to be discovered and especially microbial natural products represent promising lead-structures for antibiotic drug discovery.

Natural product  $\beta$ -lactones were shown to inhibit the eukaryotic proteasome and synthetic  $\beta$ -lactones were identified to inhibit *Staphylococcus aureus* ClpP (SaClpP)<sup>1</sup>. This motivated us to search for natural product  $\beta$ -lactones that interfere with the activity of SaClpP. ClpP, the proteolytic core of the bacterial serine protease Clp, is involved in intracellular protein turnover and regulatory proteolysis. ClpP forms a tetradecameric core-enzyme that is able to degrade small peptides. In concert with cognate Clp-ATPases, the protease complex is formed, allowing specific and tightly regulated protein degradation<sup>3</sup>.

By carrying out biochemical and microbiological analyses we were able to identify and characterize natural product inhibitors of SaClpP.

1. Böttcher & Sieber (2008). JACS; 130, 14400–14401

2. Malik & Brötz-Oesterhelt (2017). Nat Prod Rep; 34, 815 – 831

---

#### 404-MPP

Integrative action of cell-wall binding proteins of *Streptococcus pneumoniae* on the interaction with the host's immune system

\*C. Vilhena<sup>1</sup>, S. Du<sup>1</sup>, S. Hammerschmidt<sup>2</sup>, M. Rohde<sup>3</sup>, C. Skerka<sup>1</sup>, P. Zipfel<sup>1,4</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology Hans Knoell Institute, Department of Infection Biology, Jena, Germany

<sup>2</sup>Interfaculty Institute for Genetics and Functional Genomics, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

<sup>3</sup>Helmholtz Center for Infection Research, Central Facility for Microscopy, Braunschweig, Germany

<sup>4</sup>Friedrich Schiller University, Department of Microbiology, Jena, Germany

*Streptococcus pneumoniae* is a Gram-positive opportunistic pathogen that colonizes the upper respiratory tract. It is a leading cause in a wide range of infections, including community acquired pneumonia, meningitis, otitis media and bacteraemia. The pneumococcus causes 1–2 million deaths per year, most of which occur in developing countries, where this bacterial species is probably the most important pathogen of early infancy. In young children *S. pneumoniae* can also cause Hemolytic uremic syndrome (HUS), a fairly under-diagnosed kidney disease which is characterized by the triad acute kidney injury, microangiopathic hemolytic anemia, and thrombocytopenia. We collected and characterize *S. pneumoniae* strains from children with HUS in order to understand the mechanisms underlying the establishment of pneumococcus induced HUS (pHUS).

We focus on choline-binding proteins (CBPs), e.g. LytA, LytB, LytC, CbpD, PspC and PspA, and their integration and interaction on the cell wall of *S. pneumoniae*. These surface exposed pneumococcal proteins are in direct contact with the host cells and have diverse functions. The pneumococcal proteins bind host plasma proteins, participate on the building up of the pneumococcal cell wall by promoting peptidoglycan synthesis; they also play a role on cell division and on the lytic phase of this organism.

The genes derived from *S. pneumoniae* strains isolated from HUS patients were sequenced and both the domain structure and cell wall integration were compared. HUS causing *S. pneumoniae* express a certain set of Cbp and PspA variants. Proteins with related choline binding surface anchors derived from single isolates have varying numbers of choline binding units. Thus the number of choline binding units is independent from cell wall and capsule composition and likely affect the strength of cell wall integration. These clinical isolates of HUS pneumococci efficiently bound human plasminogen. Hence, HUS pneumococci damage endothelial cells in the blood vessels and disturb local complement homeostasis. Thereby, HUS pneumococci promote a thrombogenic state that drives HUS pathology.

#### 405-MPP

*Staphylococcus aureus* *farR*-mutation mediates resistance to rhodomyrtone (Rom) and causes hypervirulence

\*L. Huang<sup>1</sup>, S. H. Fan<sup>1</sup>, F. Götz<sup>1</sup>, M. Maier<sup>2</sup>

<sup>1</sup>Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Microbial Genetics, Tübingen, Germany

<sup>2</sup>Institut für Organische Chemie, Tuebingen, Germany

Rhodomyrtone (Rom) is an acylphloroglucinol antibiotic initially isolated from the leaves of *Rhodomyrtus tomentosa*. Rom is active against an extensive range of Gram-positive bacteria. Rom does not exhibit any of the classical antibiotic targets but interacts with the cytoplasmic membrane and

respiration. However, the exact mode of action of Rom is still unknown. A highly Rom resistant *Staphylococcus aureus* mutant (Rom<sup>R</sup>) could be isolated several passages of growth in the presence of Rom. Comparative genome sequencing and transcriptome analysis was performed to further characterize the Rom<sup>R</sup> mutant. The cytotoxicity of the Rom<sup>R</sup> mutant was evaluated in three human cell lines while the pathogenicity was assessed using a mouse infection model. Whole genome sequencing revealed that the Rom resistance is due to a single point mutation in the coding region of *farR*. This mutation results in an amino acid change from Cys to Arg at position 116 of FarR, thus affecting its activity. Comparative transcriptome analysis showed that the transcription of many genes involving distinct pathways was influenced by the mutated *farR*. FarR represses the expression of its own gene as well as its flanking gene *farE*, an effector of fatty acid resistance. Global regulators like *agr* and *sarA* were also repressed. Accordingly, all these genes were upregulated in the Rom<sup>R</sup> mutant. The upregulation of *agr* and *sarA* resulted in a higher expression of virulence genes, which is most likely responsible for the increased cytotoxicity and pathogenicity of the Rom<sup>R</sup> mutant. The Rom resistance is mainly attributed to the derepression of *farE*, which is described as an efflux pump for linoleic and arachidonic acids. In this regard, an increased release of lipids in the Rom<sup>R</sup> mutant compared to HG001 was observed. In conclusion, this study shows that the high Rom resistance is mediated by *farE* overexpression in the Rom<sup>R</sup> mutant. FarR appears to be a global regulator that decreases virulence gene expression. Mutation in *farR* rendered the clone hypervirulent (Rom<sup>R</sup> mutant).

#### 406-MPP

The role of a putative phosphoethanolamin transferase (PA\_21210) in *Pseudomonas aeruginosa* PA14

\*J. Rehner<sup>1</sup>, S. Zehner<sup>1</sup>, K. Gerbracht<sup>1</sup>, N. Frankenberg-Dinkel<sup>1</sup>

<sup>1</sup>Technische Universität Kaiserslautern, Mikrobiologie, Kaiserslautern, Germany

Lipopolysaccharides (LPS) are the major constituents of this outer membrane of Gram-negative bacteria. They consist of three different domains: lipid A, which is anchored in the outer membrane and acts as an endotoxin if the membrane is destroyed, an inner core sugar region and an outer polysaccharide, also known as O-antigen. Lipid A carries a negative charge which can be targeted by cationic antimicrobial peptides that lead to cell lysis due to a destabilization of the outer membrane. A modification of lipid A molecules by addition of phosphoethanolamine (pEtN) leads to an increase in polymyxin resistance by functioning as a shield for the negatively charged lipid A. pEtN-transferases (EptA) are catalyzing this reaction and can be found in Gram-negative bacteria such as *E. coli*, *Neisseria meningitidis* and *Salmonella enterica*. Three orthologs of this enzyme are found in the opportunistic pathogen *Pseudomonas aeruginosa* PA14. While for two of them, a pEtN-transferase activity was confirmed, the protein encoded by PA\_21210 was inactive. Interestingly, a pEtN-transferases from *Campylobacter jejuni* was shown to modify the FlgG subunit of flagella, while a homolog from *Neisseria gonorrhoeae* modifies type IV pili. Within the study presented here, we wish to explore the function of the putative pEtN-transferases encoded by PA\_21210 in *P. aeruginosa* PA14. Our study will initial focus on the phenotypic investigation of a markerless chromosomal knock-out mutant. Focus will be on motility and biofilm assays as these either rely on functional flagella and/or type IV pili. Once an effect is observed, the respective structure will be isolated and tested for modification using mass spectrometry.

#### 407-MPP

The author has not agreed to a publication

#### 408-MPP

##### Investigating the effect of antimicrobial peptides on type III secretion kinetics

\*S. Zilkenat<sup>1</sup>, S. Wagner<sup>1</sup>

<sup>1</sup>Interfaculty Institute of Microbiology and Infection Medicine  
Tübingen, Cellular and Molecular Microbiology, Tübingen, Germany

The type III secretion system is a major virulence factor expressed by many gram-negative pathogens, such as *Escherichia*, *Shigella*, *Salmonella* and *Yersinia* species. The system is energized by ATP hydrolysis and the proton motive force of the inner membrane. Bacteria use it to translocate effector proteins across their own cell membranes and into the host cell. It has been studied in detail in different organisms, however, a number of important questions remain unanswered due to the inability to directly assess the effects on secretion kinetics.

In our lab, we recently developed a NanoLuc luciferase-based type III secretion assay<sup>[1]</sup>. This method gives us the ability to measure type III secretion and injection robustly and quickly. Now we are advancing this approach by integrating it into a newly developed microfluidic device, designed to monitor bacterial growth by fluorescence microscopy and to rapidly introduced compounds to the bacteria, while also collecting flow through fractions. Luminescence measurement of the corresponding fractions will allow us to directly record sudden changes in secretion upon perturbation due to these compounds. At the same time membrane potential dyes and specific fluorophore tags enable us to directly track the behavior of cells during the perturbation.

Once established, we are using the device to track the effects of e.g. antimicrobial peptides, cholic acids, or antibiotics on the secretion kinetics of *Salmonella* Typhimurium.

-

[1] Sibel Westerhausen, Melanie Nowak, Claudia Torres-Vargas, Ursula Bilitewski, Erwin Bohn, Iwan Grin, Samuel Wagner. 2019. A NanoLuc luciferase-based assay enabling the real-time analysis of protein secretion and injection by bacterial type III secretion systems. bioRxiv 745471

#### 409-MPP

##### Impact of the transcription factor SpoVG on biofilm formation of *Staphylococcus epidermidis* *in vitro* and *in vivo*.

\*H. Benthien<sup>1</sup>, B. Jonas<sup>2</sup>, L. Pätzold<sup>1</sup>, M. I. Elhawy<sup>1</sup>, S. Becker<sup>1</sup>, \*M. Bischoff<sup>1</sup>, J. Knobloch<sup>2</sup>

<sup>1</sup>Saarland University, Medical Microbiology and Hygiene, Homburg, Germany

<sup>2</sup>University Medical Center Hamburg-Eppendorf, Medical Microbiology, Virology and Hygiene, Hamburg, Germany

*Staphylococcus epidermidis* is a major cause of implantable device related infections. The opportunistic pathogen is capable of forming biofilms on implantable devices, a process that usually involves the formation of an extracellular matrix (ECM). Most clinical *S. epidermidis* isolates form an ECM that includes the formation of an exopolysaccharide called polysaccharide intercellular adhesin (PIA), which production requires the factors encoded by the *ica* operon. Transcription of the *ica* operon is under multiple levels of control in *S. epidermidis*, and involves the alternative

transcription factor  $\sigma^B$ . However, since the promoter region of the *ica* operon lacks a clear  $\sigma^B$  consensus sequence, the impact of this sigma factor on *ica* expression is likely to be indirect.

Here we show that the strictly  $\sigma^B$ -dependent transcription factor SpoVG affects transcription of the *ica* operon and biofilm formation of *S. epidermidis*. Deletion of *spoVG* in the PIA producing isolate SE1457 reduced the transcription of *icaA* in a similar way as an isogenic *sigB* mutant, without markedly affecting the transcription of the repressor of this operon, encoded by *icaR*. The SE1457  $\Delta$ *spoVG* mutant also displayed a drastically reduced PIA production, and a strongly decreased biofilm formation in microtiter plate-based assays and on catheter fragments under *in vitro* growth conditions. Both findings suggest that SpoVG might be important for pathogenesis of PIA producing *S. epidermidis*. However, when SE1457 and its isogenic *spoVG* mutant were tested in a murine foreign body related infection model, only minor differences in bacterial loads on the implanted catheter fragments and the surrounding tissues were detected between wild-type and *spoVG* mutant challenged mice, although SE1457  $\Delta$ *spoVG* infected mice displayed a significantly reduced edema formation at the infection site. These findings indicate that SpoVG, albeit of being of major importance for PIA production and biofilm formation of *S. epidermidis* under *in vitro* conditions, is largely dispensable for biofilm formation on implantable devices under *in vivo* conditions, but does affect the inflammatory response of the host at the infection site.

#### 410-MPP

##### The FlhC polyproline motif is essential for flagellar motility in *Salmonella* Typhimurium

\*C. Kühne<sup>1</sup>, I. Spöring<sup>2</sup>, K. Jung<sup>3</sup>, M. Erhardt<sup>1,2</sup>

<sup>1</sup>Humboldt-Universität zu Berlin, Institute of Biology, Berlin, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

<sup>3</sup>Ludwig-Maximilians-Universität München, Department of Biology, Martinsried, Germany

**Question:** Flagella-mediated motility plays a crucial role for *Salmonella* pathogenicity by enabling directed movement. Flagella synthesis is tightly regulated by a transcriptional hierarchy. The flagellar master regulatory protein complex FlhD<sub>4</sub>C<sub>2</sub> initiates transcription of genes required for assembly of the bacterial flagellum. The FlhC subunit harbors a stretch of three consecutive proline residues, called a polyproline motif. This motif is abundant in proteins, but generally slows down translation due to ribosome stalling, which needs to be alleviated by the elongation factor EF-P. The functionality and role of the polyproline motif in the flagellar protein FlhC is poorly understood. Here, we characterized the role of the FlhC polyproline motif for flagellar motility in *Salmonella* Typhimurium.

**Methods:** We generated a set of FlhC polyproline mutants by replacing proline residues with alanine and analyzed their effects on flagellar motility, the transcriptional hierarchy and FlhC protein stability. Further, we performed a random mutagenesis screen followed by whole-genome-sequencing to identify bypass mutations in a FlhC<sub>AAA</sub> mutant.

**Results:** We found that the FlhC polyproline motif is essential for flagellar motility. We observed a motility defect and down-regulation of flagellar gene expression for all polyproline mutants, which was dependent on number and position of the exchanged prolines, but strongest for the FlhC<sub>AAA</sub> mutant. Also, degradation of FlhC protein was highly enhanced in FlhC<sub>AAA</sub>, which could be compensated by additional deletion of the proteases Lon and ClpXP. A random mutagenesis screen for motility suppressors of the

Flh<sub>CAA</sub> mutant revealed *flhDC* promoter mutations, which presumably increased *flhDC* transcription and bypassed the increased degradation of FlhC.

**Conclusions:** Here, we characterize the role of the conserved polyproline motif in the flagellar master regulatory protein FlhC. Our results suggest that the motif is necessary for controlling the correct copy number of the FlhD4C2 complex by controlling translation speed and folding of FlhC.

---

#### 411-MPP

Monoclonal antibodies against Shiga Toxin 2a uncovers different subunits by proteolytic cleavage with Furin and Trypsin

\*M. Grasse<sup>1</sup>, M. O. Skjold<sup>2</sup>, S. Kellnerova<sup>1</sup>, D. Orth-Höller<sup>1</sup>, R. Würzner<sup>1</sup>

<sup>1</sup>Medical University of Innsbruck, Hygiene and Medical Microbiology, Innsbruck, Austria

<sup>2</sup>University Hospital of Copenhagen, Rigshospitalet, Clinical Immunology and Tissue Typing Lab/Hybridoma Center, Copenhagen, Denmark

**Objectives:** Enterohemorrhagic *Escherichia coli* (EHEC) infections are a major cause of EHEC-associated Haemolytic Uraemic Syndrome (eHUS). Shiga toxins (Stxs) are thought to play a major role in EHEC pathogenesis. Stxs are released into the intestinal lumen and translocate into the circulation to reach their target organs, mainly kidneys and brain. Stx2a is an AB5 holotoxin that consists of one enzymatically active A subunit, which is noncovalently associated with a pentameric B subunit. The A subunit can occur in two fragments after proteolytic cleavage, A1 and A2 subunits. However, it is still unknown where and how the A subunit is cleaved.

**Methods:** Peptide immunization strategy in outbred mice with PEG fusion of SP2 cells and splenocytes was applied to design 3 monoclonal antibodies (mAb), aiming to detect either the C-terminal part of Stx2a-A1, the N-terminal part of Stx2a-A2, or the uncleaved Stx2a-A subunit. Western Blots and enzyme-linked immunosorbent assays (ELISAs) were performed with Stx2a that was either digested with Trypsin or Furin, and with untreated Stx2a, to test the binding properties of the mAbs. Furthermore, the Trypsin- and Furin-digested Stx2a was analyzed for amino acid (AA) sequence by mass spectrometry.

**Results:** Western blot analysis showed one mAb detecting the whole Stx2a-A subunit and several candidates of mAbs detecting either Stx2a-A1, -A2 when the toxin was digested with Trypsin. Stx2a digested with Furin couldn't be detected with the mAbs. The ELISAs revealed, that the mAbs can only detect the denaturated Stx2a, but not the native form. The mass spectrometry analysis showed, that Stx2a digestion by Trypsin and Furin result in Stx2a-A1 and -A2 subunits that differ in a 3 AA shifted cleavage site.

**Conclusion:** Stx2a is digested in vitro differently by the human physiologically enzymes Trypsin (extracellular) and Furin (intracellular). It needs to be elucidated, if this is happening also in the in vivo situation and what functional consequences arise for the Stx2a-A1 and -A2 subunits. The mAbs for detecting Furin-digested Stx2a need to be designed differently than the ones for Trypsin-digested Stx2a.

---

#### 412-MPP

Pneumococcal pneumolysin modulates platelet functionality

\*K. Jahn<sup>1</sup>, S. Handtke<sup>2</sup>, R. Palankar<sup>2</sup>, T. Kohler<sup>1</sup>, J. Wesche<sup>2</sup>, M.

Rohde<sup>3</sup>, A. Greinacher<sup>2</sup>, S. Hammerschmidt<sup>1</sup>

<sup>1</sup>University of Greifswald, Molecular Genetics and Infection Biology, Greifswald, Germany

<sup>2</sup>University Medicine Greifswald, Transfusion Medicine, Greifswald, Germany

<sup>3</sup>Helmholtz Centre for Infection Research, Braunschweig, Central Facility for Microscopy, Braunschweig, Germany

#### Introduction

*Streptococcus pneumoniae* is a commensal of the upper airways but can also infiltrate into sterile niches of the body and is responsible for severe infectious diseases, including pneumonia, meningitis and sepsis. During infection, pneumococci are able to enter the blood stream and interact with platelets. The activation and aggregation of platelets during bacteremia is of high clinical importance, since septic patients often develop thrombocytopenia and a low platelet count correlates with an increased morbidity. Pneumolysin (Ply), a pore-forming cholesterol-dependent cytolysin and a major virulence factor of *S. pneumoniae*, has earlier been shown to activate platelets. The aim of this project was to assess systematically the impact of pneumococcal Ply on platelet activation and functionality.

#### Methods

Platelet activation and function was measured using flow cytometry with various activation markers, as well as by light transmission aggregometry. Furthermore, Ca<sup>2+</sup> release and platelet viability was determined. Thrombus formation was assessed in a flow chamber with hirudinated blood.

#### Results

Ply induces pore formation in platelet membranes, leading to a loss of platelet viability in a concentration dependent manner. Measurement of platelet activation via integrin activation revealed no increase in signal intensity. The previous reported platelet activation via measurement of CD62P surface expression was also shown, however, due to pore formation, the antibody stains intracellular CD62P. After Ply treatment TRAP-6 was added as a test for platelet functionality. Only after incubation with sublytic Ply concentrations, platelets reacted to TRAP-6 stimulation. Also in whole blood, platelet function was significantly diminished after Ply treatment.

#### Conclusion

Ply perforates platelet membranes and induces a rapid loss of functionality and viability of platelets. The loss of platelet function even at low Ply concentrations might be a main contributor to capillary leakage in severe pneumococcal pneumonia.

---

#### 413-MPP

Disentangling the role of *Staphylococcus aureus* in skin dysbiosis

\*Z. Wang<sup>1</sup>, C. Hülpmich<sup>2</sup>, V. Schwierzeck<sup>2</sup>, M. Reiger<sup>2</sup>, C. Treidl-Hoffmann<sup>2</sup>, M. Schlöter<sup>3</sup>, B. Foessel<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München, Comparative Microbiome Analyses, München, Germany

<sup>2</sup>Technical University of Munich, Institute of Environmental Medicine, UNIKA-T, Augsburg, Germany

<sup>3</sup>Technical University of Munich, Central Institute for Food and Health (ZIEL), Freising, Germany



Host-microbe interactions play a crucial role in the onset of atopic dermatitis (AD). Skin barrier dysfunction and microbiome dysbiosis are a hallmark of AD pathogenesis. *Staphylococcus aureus* is the most problematic pathogen of the genus causing numerous acute and chronic infections. It has been shown that skin dysbiosis in AD is characterized by reduction of microbial diversity, accompanied by an increase in *S. aureus* abundance, as well as *S. aureus* exo- and enterotoxin concentrations. A recent analysis of microbiome-transcriptome correlations shows that dysbiosis of various *Staphylococcus* species in AD is associated with skin dysregulation. However, it is still not clear whether *S. aureus* colonization is a cause of skin dysfunction or the driver of AD. Furthermore, the role of different *S. aureus* strains remains to be differentiated.

Here a set of 48 *S. aureus* strains from skin and nose of AD patients as well as the nose of healthy individuals (control) was isolated and their identity verified by MALDI-TOF analysis; 16S rRNA gene analysis confirmed species identity. However, further phylogenetic resolution beyond species level is not possible using this approach. In contrast, first physiological tests using the API® ID 32 STAPH and 50 CH series already indicate strain-level differentiation according to health status and individuals derived from. We performed a comparative genomics approach to characterize the strains beyond the species level and to provide a functional description. Genes and pathways related to metabolic differences for the isolates have been identified and linked to physiological testing.

This will provide insight into the role of different *S. aureus* strains in skin dysbiosis and the mechanism of epidermal barrier dysfunction and dysbiosis. Moreover, the knowledge obtained could provide potential novel therapeutic approaches in AD and other atopic diseases.

---

#### 414-MPP

Characterization of an *Escherichia coli* small colony variant descended from ABU strain 83972 during deliberate human bladder colonization

\*J. Putze<sup>1</sup>, O. Mantel<sup>1</sup>, U. Dobrindt<sup>1</sup>

<sup>1</sup>Universitätsklinikum Münster, Institut für Hygiene, Münster, Germany

**Introduction** Bacterial small colony variants (SCVs) grow slow and form tiny colonies compared to other strains of the same species. The observation of SCVs is reported since 1910 for several bacterial species including *S. aureus*, *P. aeruginosa*, *Salmonella* sp., *E. coli* and others. While most of the reports describe staphylococcal SCVs and SCVs of *Pseudomonas*, only comparatively few records of *E. coli* SCVs are published. Similarly, knowledge of the biological meaning, importance in disease and the underlying molecular mechanisms of *E. coli* SCVs is scarce.

**Objective** We sought to find the molecular reason of SCV formation and to unravel potential bacterial evolutionary adaptations leading to the identified SCV phenotype during colonization of the human bladder.

**Materials & Methods** Bacteria were cultivated on solid or in liquid lysogeny broth and pooled human urine. Construction of mutants and complementation were done by standard methods. The adhesive, invasive and intracellular survival abilities were determined using human urinary bladder (T24) and kidney epithelial (A-498) cells. Whole genome sequencing (WGS) was done by Illumina sequencing. Growth kinetics were recorded in a microplate reader.

**Results** We verified the identity of the SCV isolate as a descendant of 83972 by WGS. Sequence analysis further revealed 13 non-synonymous SNPs and one synonymous SNP as well as three deletions and two insertions. The highest impact have probably three frameshift variants in *cysB*, *srnB* and *gidA* as well as a stop gain in *rsd*. Indeed, deletion as well as complementation of these genes resulted in variable strong effects on growth of mutants as well as of the original SCV isolate, respectively. The growth defects of the original SCV isolate and the constructed single- and multiple gene mutants were striking when analyzed using lysogeny broth. However, when we used pooled human urine as a more "natural" medium, the differences in growth were much less pronounced. *In vitro*, we could not detect spontaneous reversion to wild-type growth and no obvious advantage compared to the ancestor 83972.

**Conclusion** We identified several genes, which can cause or provoke a SCV phenotype.

---

#### 415-MPP

Deciphering the role of the CRISPR/Cas system in *Neisseria meningitidis* in host cell adhesion

\*D. Müller<sup>1</sup>, S. Bauriedl<sup>1</sup>, C. Schoen<sup>1</sup>

<sup>1</sup>Institut für Hygiene und Mikrobiologie, Universität Würzburg, Würzburg, Germany

**Introduction:** *Neisseria meningitidis* (Nm) is a commensal of the human nasopharynx and a worldwide leading cause of sepsis and meningitis. The meningococcal CRISPR/Cas locus contains a CRISPR array with an extended -10 box within each spacer region, a tracrRNA, genes encoding canonical Cas1, Cas2 and Cas9 proteins as well as an uncharacterized small non-coding RNA NMnc0040 [1].

We could recently show that the CRISPR/Cas system in Nm serogroup C strain 8013 is important for the adhesion to human nasopharyngeal cells [2]. In particular, Cas9 knock-out strains were impaired in the adhesion to host cells probably via affecting the expression of a small basic lipoprotein Blp along with NMnc0040.

**Objectives:** Here, we want to address whether and how Blp and NMnc0040 affect meningococcal adhesion to human nasopharyngeal cells.

**Methods:** To validate the expression profile of NMnc0040 and Blp we performed northern blots analyses in different growth conditions. We further generated *blp* and *NMnc0040* knock-out strains and isogenic complemented strains and used these strains in adhesion assays with human nasopharyngeal cell lines.

**Results:** Northern Blot analyses showed that NMnc0040 is constitutively expressed. Adhesion assays further showed that, in contrast to Cas9 and tracrRNA, deletion of NMnc0040 or Blp increased the adhesion of meningococci to human cells. Of note, a double knock-out strain lacking Cas9 and Blp showed wild-type adhesion rate, indicating that Blp acts as an intergenic suppressor of Cas9.

**Conclusion:** Our preliminary genetic data suggest that Cas9/tracrRNA and Blp/NMnc0040 interact and have opposing effects on meningococcal host cell adhesion probably via affecting the function and/or expression of yet to identify adhesion molecules.

[1] Zhang Y et al., Mol Cell, 2013, 50(4):488-503 [2] Heidrich N et al., RNA Biology, 2018, 16(4):390-396

---

#### 416-MPP

##### Role of the *comE* operon in *Staphylococcus lugdunensis* biofilm formation

\*T. Grebe<sup>1</sup>, C. Rumpf<sup>1</sup>, \*C. Heilmann<sup>1</sup>

<sup>1</sup>University Hospital Münster, Institute of Medical Microbiology, Münster, Germany

**Introduction:** Coagulase-negative staphylococci (CoNS) can cause life-threatening infections, such as infective endocarditis and sepsis frequently associated with the use of medical devices. The main pathogenicity factor in CoNS infection is biofilm formation.

**Objectives:** In *Staphylococcus lugdunensis*, beside proteins extracellular (e)DNA is a major structural component of the biofilm matrix. By transposon mutagenesis, we identified a novel, lysis-independent mechanism of eDNA generation, which involves the competence gene *comEB*. *comEB* is part of the *comEABC* operon, whose function in staphylococci is still unclear. Its homolog in *Bacillus subtilis* encodes the DNA uptake machinery involved in natural competence. Thus, we hypothesize that *S. lugdunensis* has converted the regular functions of competence genes resulting in the secretion of DNA rather than the uptake of DNA.

**Materials and Methods:** To characterize the function of the *comEABC* operon, we constructed a *comEABC* deletion mutant, which subsequently was complemented by cloning the whole *comEABC* operon. To study the involvement of each *comE* gene in biofilm formation, single and double site-directed *comEABC* deletion mutants were constructed by inverse PCR. Biofilm formation was analysed in a quantitative biofilm assay performed in microtiter plates.

**Results:** The *comEABC* mutant exhibited a similarly reduced biofilm-forming capacity as previously observed with the *comEB* transposon mutant. The cloned *comEABC* operon was able to complement the *comEABC* mutant to the wild-type level of biofilm formation. Biofilm assays of site-directed *comEA*, *comEB*, and/or *comEC* single and double mutants revealed that only clones with an intact *comEB* gene formed biofilms at wild-type levels independently of the presence of *comEA* and *comEC*.

**Conclusion:** The functions of *ComEA* and *ComEC*, which are hypothesized to build the membrane channel in DNA secretion, may be dispensable in biofilm eDNA generation, potentially due to gene duplications or due to the use of an alternative DNA transport system. Further analyses are on the way to address this question and to study DNA secretion and subcellular location of *ComE* proteins.

---

#### 417-MPP

##### The role of the *nmpC* and *yidR* genes in the virulence of *Salmonella* Enteritidis to intestinal epithelial cells

\*M. Wimonć<sup>1</sup>, R. Kolenda<sup>1</sup>, M. Ugorski<sup>1</sup>

<sup>1</sup>Wrocław University of Environmental and Life Sciences, Department of Biochemistry and Molecular Biology, Wrocław, Poland

#### Introduction

*Salmonella* Enteritidis is currently one of the major intestinal pathogens. Adhesion to and invasion of host cells is one of the first critical steps in the pathogenesis of *Salmonella* infections. An important process is identification and knowledge of the function of genes associated with colonization of the gastrointestinal tract by these pathogens.

In the studies performed were found that in the case of *S. Enteritidis* with low adhesion-invasive phenotype, *nmpC* and *yidR* genes are characterized by the presence of mutations in comparison to the genome of the reference strain P125109.

#### Objectives

Studies on the *nmpC* gene, which encodes OmpD protein, have so far only concerned *S. Typhimurium* and showed different results. On the one hand, using Int-407 cells, no differences in adhesion and invasion between wild strains and mutants with *nmpC* gene knockout were found. We know less about the *yidR* gene, which is only known to have reduced *S. adhesion*. *Typhimurium* to lettuce leaves. The role of *nmpC* and *yidR* genes in adhesion to and invasion of *S. Enteritidis* into epithelial cells is poorly understood, therefore the aim was to explain the role of these genes in the pathogenesis of *S. Enteritidis* infections.

#### Materials & methods

In order to remove the tested genes and replace them with antibiotic resistance genes, a homologous recombination according to the Datsenko and Wanner protocols was applied. Adhesion and invasion tests were performed using three lines of intestinal epithelium of human (Caco-2).

#### Results

Each of the strains was examined in terms of morphology - shape and formation of aggregates and growth rate. The study did not show any differences in morphology. Adhesion and invasion tests performed on strains with different variants of the *nmpC* and *yidR* genes have shown that wild strains of these genes cause higher adhesion and invasion in *Salmonella* Enteritidis.

#### Conclusion

Adhesive and invasive tests have shown that selected genes can affect *Salmonella* Enteritidis virulence. However, in order to fully understand the basic mechanisms involved in the infection of host cells with *Salmonella*, more experiments need to be conducted.

---

#### 418-MPP

##### *Klebsiella pneumoniae* in porcine blood: bactericidal activity of adaptive IgM and proline-rich antimicrobial peptides

\*A. K. Krieger<sup>1</sup>, T. Ludwig<sup>2</sup>, A. Krizsan<sup>2</sup>, I. Eder<sup>3</sup>, S. Öhlmann<sup>1</sup>, L. Mayer<sup>1</sup>, G. Köller<sup>4</sup>, R. Hoffmann<sup>2</sup>, C. Baums<sup>1</sup>, K. Rieckmann<sup>1</sup>

<sup>1</sup>Faculty of Veterinary Medicine Leipzig, Institute of Bacteriology and Mycology, Leipzig, Germany

<sup>2</sup>University of Leipzig, Center for Biotechnology and Biomedicine, Leipzig, Germany

<sup>3</sup>University Medicine of Leipzig, Germany, Medical Microbiology and Epidemiology of Infectious Diseases, Leipzig, Germany

<sup>4</sup>Faculty of Veterinary Medicine Leipzig, Department of Ruminants and Swine, Leipzig, Germany

Multidrug-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) is considered a major global concern by the World Health Organization. Infections with hypervirulent *K. pneumoniae* strains may lead to severe symptoms including death in association with the liver abscess syndrome. *K. pneumoniae* is also an emerging pathogen in piglets causing septicemia.

Here, bactericidal assays with freshly drawn porcine blood were evaluated for investigating *K. pneumoniae* bacteremia *in vitro*, including comparison of strains, development of immunity and bactericidal activity of antimicrobial peptides (AMP). Specifically, we investigated the hypothesis that proliferation of invasive *K. pneumoniae* in porcine blood might be inhibited by proline-rich AMPs.

Survival of *K. pneumoniae* in porcine blood exhibited differences between strains in association with the clinical background of the strain. Through addition of the specific IgM protease rIdcS<sub>suis</sub> we showed that IgM plays an important role in the early adaptive immune response leading to killing of an invasive *K. pneumoniae* strain in blood of 8 week old piglets. Addition of proline-rich AMPs reduced the survival of *K. pneumoniae* in porcine blood of younger piglets significantly and in a concentration dependent manner. As evaluated by lactate dehydrogenase release, cytotoxicity of the tested proline-rich AMPs was rather low and comparable to the cephalosporin cefquinom. Induction of proinflammatory cytokines was determined through measurements of TNF- $\alpha$ . Results showed large differences in TNF- $\alpha$  induction between individual piglets and no significant difference in assays with cefquinom or AMP treatment.

In summary, we established the bactericidal assay with porcine blood as a novel *in vitro* method to evaluate the bactericidal, cytotoxic and immunogenic activity of AMPs in *K. pneumoniae* bacteremia and show the first promising data for proline-rich AMPs.

#### 419-MPP

##### D-Alanylation of Lipoteichoic Acids as Putative Immune Evasion Mechanism of *Streptococcus suis*

\*S. Oehlmann<sup>1</sup>, M. Meurer<sup>2</sup>, N. de Buhr<sup>2</sup>, A. K. Krieger<sup>1</sup>, N. Gisch<sup>3</sup>, D. Knappe<sup>4</sup>, R. Hoffmann<sup>4</sup>, M. von Kockritz-Blickwede<sup>2</sup>, C. G. Baums<sup>1</sup>

<sup>1</sup>Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, University of Leipzig, Germany, Leipzig, Germany

<sup>2</sup>Research Center for Emerging Infections and Zoonoses (RIZ), University of Veterinary Medicine Hannover, Department of Physiological Chemistry, Hannover, Germany

<sup>3</sup>Division of Bioanalytical Chemistry, Priority Area Infections, Research Center Borstel, Leibniz Lung Center, Germany, Borstel, Germany

<sup>4</sup>Center for Biotechnology and Biomedicine, University of Leipzig, Germany, Leipzig, Germany

*Streptococcus suis* (*S. suis*) is a major pathogen in piglets that leads to high economic losses in the swine industry by causing septicemia, meningitis, arthritis, endocarditis and pneumonia. As a zoonotic agent it has so far been responsible for more than 1600 human infections worldwide.

As in other Gram-positive bacteria, lipoteichoic acids (LTAs) are main constituents of the cell wall of *S. suis* [1]. Their D-alanylation is described as a bacterial evasion mechanism against killing by neutrophilic granulocytes and antimicrobial peptides (AMPs) [2]. In *Streptococcus pneumoniae* adding D-alanine to LTA was suggested to increase the inflammatory response of the host resulting in more efficient shedding and transmission of bacteria [3].

Since bacteremia is a hallmark of invasive *S. suis* infections, we investigated the influence of D-alanylation of LTA on the bacterial survival in porcine and human blood, as well as its capacity to modulate the inflammatory response of blood leukocytes.

A mutant deficient in the D-alanylation of LTA was generated in order to perform loss-of-function experiments. We evaluated the survival and the induced cytokine production, tested the influence of proline-rich AMPs in bactericidal assays and determined the minimal bactericidal concentrations of a number of AMPs.

The loss of the ability to D-alanylate LTA in the isogenic mutant was confirmed by nuclear magnetic resonance. In accordance with previous studies, we observed an increase in susceptibility to AMPs in our deletion mutant. Nevertheless, D-alanylation of LTA was not crucial for survival in porcine or human blood in our set-up.

[1] Gisch et al., Journal of Biological Chemistry 2018, 293(31): 12011-12025

[2] Fittipaldi et al., Infection and Immunity 2008, 76 (8): p3587 - 3594

[3] Zafar et al., mBio 2019, 10:e01032-19

#### 420-MPP

##### The *Acinetobacter* Trimeric Autotransporter Adhesin Ata drives the Pathogenicity of *Acinetobacter baumannii* and modulates the Host Cell Response

\*M. Tietgen<sup>1</sup>, S. Wohra<sup>1</sup>, M. Weidensdorfer<sup>1</sup>, I. Wittig<sup>2</sup>, P. Kraiczyn<sup>1</sup>, V. A. J. Kempf<sup>1</sup>, S. Göttig<sup>1</sup>

<sup>1</sup>Institute for Medical Microbiology and Infection Control, University Hospital, Goethe University, Frankfurt am Main, Germany

<sup>2</sup>Faculty of Medicine, Functional Proteomics, Goethe University, Frankfurt am Main, Germany

**Introduction:** Trimeric autotransporter adhesins (TAAs) are important virulence factors in many Gram-negative bacteria. The *Acinetobacter* trimeric autotransporter adhesin Ata is the TAA of *Acinetobacter baumannii*, one of the most clinically significant pathogens.

**Aim:** To analyze the impact of Ata on the host cell response during infection, human umbilical cord vein endothelial cells (HUVECs) were infected with *A. baumannii* ATCC 19606 (WT) or an isogenic *ata* knock-out mutant ( $\Delta$ ata).

**Methods:** Quantitative proteomics using liquid chromatography/mass spectrometry was carried out to analyze protein expression of HUVECs 14 h post infection. Chemokine secretion was analyzed using Multi-Analyte ELISArray Kits (Qiagen). Chemotaxis of human THP-1 monocytes was investigated employing a transmigration assay. Recruitment of immune cells *in vivo* was evaluated using the *Galleria mellonella* infection model. Induction of apoptosis was determined using propidium iodide/annexin V staining followed by FACS analysis and the Caspase-Glo<sup>®</sup> 3/7 assay (Promega).

**Results:** In total 4,969 human proteins were quantified, from which 106 (WT) and 51 ( $\Delta$ ata) proteins were differentially produced (fold change  $\geq$  2; FDR < 0.05). Two signaling pathways involved in inflammation/immune response and induction of apoptosis were identified and further verified in infection experiments. Upon infection of HUVECs with *A. baumannii*, interleukin-6 (IL) and IL-8 were secreted in a time- and Ata-dependent way. Likewise, chemotaxis of THP-1 cells towards HUVECs was significantly increased upon infection with the WT compared to  $\Delta$ ata. After injection into *Galleria mellonella* larvae, *ata* deletion mutants were attenuated in their ability to recruit immune cells *in vivo*. Higher apoptosis rates were measured upon infection of

HUVECs with the WT compared to  $\Delta$ ata which were dependent on cleavage of caspase-3 and caspase-7.

**Conclusion:** Ata caused a pro-inflammatory host cell response and induced apoptosis by activation of caspase-3/-7. These results suggest that Ata is an important and multifunctional virulence factor of *A. baumannii*.

#### 421-MPP

Myo-inositol as a carbon substrate in *Francisella* and insights into the metabolism of *Francisella* sp. strain W12-1067

\*K. Köppen<sup>1</sup>, F. Chen<sup>2</sup>, K. Rydzewski<sup>1</sup>, W. Eisenreich<sup>2</sup>, K. Heuner<sup>1</sup>

<sup>1</sup>Robert Koch-Institut, Berlin, Germany

<sup>2</sup>Technische Universität München, München, Germany

*Francisella tularensis* (Ft) is an intracellular pathogen causing tularemia in a variety of hosts including humans and rodents. Various transmission and infection routes have been described causing different clinical manifestation like massive swollen lymph nodes and pneumonia. So far, all reported cases of tularemia in Germany have been caused by the Ft subspecies *holarctica* (Fth). However, a new environmental *Francisella* sp. strain W12-1067 (F-W12) not belonging to Fth was found. This isolate does not possess the *Francisella* pathogenicity island but *in silico* analysis revealed a putative alternative type VI secretion system and other virulence factors in its genome. Moreover, a putative myo-inositol (MI) metabolizing gene cluster was identified *in silico* exhibiting a putative inositol oxygenase. A similar MI metabolizing gene cluster has been found in other *Francisella* strains like *Francisella novicida* (Fno) strain Fx1 but is missing in Fth LVS and Fno U112. Various bacteria are able to use MI as the exclusive carbon source and inositol oxygenase is known to convert MI into glucuronic acid and further into 3-phosphoglycerate and pyruvate. To analyze the capacity of F-W12 to metabolize MI, a MI gene cluster negative mutant was generated ( $\Delta$ MI). Growth experiments revealed that MI increased growth rates of strain F-W12 and Fno Fx1 in the absence of glucose but this was not observed for  $\Delta$ MI mutant strain and not for Fno U112. In addition, isotopologue profiling experiments confirmed the involvement of the MI gene cluster in the metabolism of MI in strain F-W12. Labeling with <sup>13</sup>C-glucose, serine or glycerol during growth in medium T revealed a bipartite metabolism in F-W12. Glucose is mainly metabolized via glycolysis but not through the Entner-Doudoroff or the pentose phosphate pathway. Carbon flux from <sup>13</sup>C-glycerol and <sup>13</sup>C-serine was less active; they were converted mostly into amino acids, lactate and fatty acids during growth in medium T. In conclusion, the metabolism of F-W12 seems to be more related to the metabolism described for Fno then for Fth, especially regarding the capacity to metabolize MI and the usage of glycerol and glucose.

#### 422-MPP

**Intracellular accumulation of trehalose-6-phosphate leads to growth inhibition in *Acinetobacter baumannii***

\*J. Hubloher<sup>1</sup>, V. Müller<sup>1</sup>

<sup>1</sup>Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt, Germany

**Introduction:** The nosocomial pathogen *Acinetobacter baumannii* accumulates trehalose upon osmotic and heat stress via the OtsA-OtsB pathway [1]. In this pathway OtsA catalyzes the condensation of glucose-6-phosphate and UDP-glucose, resulting in the formation of trehalose-6-

phosphate (T6P). In the next step OtsB cleaves off the phosphate and releases trehalose. The absence of *otsB* in *A. baumannii* leads to the inability to kill *Galleria mellonella* larvae [2], suggesting that the compatible solute is involved in pathogenicity.

**Objectives:** To challenge the hypothesis that trehalose is involved in pathogenesis.

**Materials & methods:** Markerless deletion mutants of *otsA*, *otsB* and *otsAB* and strains overproducing OtsA, OtsB and OtsAB were generated in *A. baumannii* 19606. The growth behavior of the different strains was monitored and the accumulation of T6P was measured.

**Results:** *A. baumannii* accumulates trehalose upon growth at high salt (300 mM NaCl) in minimal medium with arabinose as carbon source. The same holds true for growth at 45 °C. Deletion of *otsB* led to growth inhibition, while in contrast deletion of *otsA* and *otsAB* did not. Overproduction of OtsA also led to growth inhibition. Deletion of *otsB* or overproduction of OtsA led to the intracellular accumulation of T6P in *A. baumannii*.

**Conclusion:** From the data presented, it is obvious that not the absence of trehalose but the presence of T6P causes growth inhibition in *A. baumannii*. T6P is a key signaling metabolite during plant development [3] and sugar metabolism in yeast [4]. Furthermore, deletion of *otsB* in *Mycobacterium tuberculosis* is lethal caused by T6P accumulation. The same seems to be true for *A. baumannii*. The basis for T6P inhibition is currently under investigation.

1. Zeidler S, Hubloher J, Schabacker K, Lamosa P, Santos H, Müller V. 2017. Environ Microbiol **19**: 5088-5099

2. Gebhardt M, Gallagher L, Jacobson R, Usacheva E, Peterson L, Zurawski D, Shuman H. 2015. MBio **6**: e01660-e01615

3. Ponnu J, Wahl V, Schmid M. 2011 Front Plant Sci **2**: 70

4. Deroover S, Ghillebert R, Broeckx T, Winderickx J, Rolland F. 2016. FEMS Yeast Res **16**: fow036

#### 423-MPP

**Characterization of *Brassicaceae* smut fungal effectors responsible for plant infection**

\*S. Gul<sup>1</sup>, N. Heßler<sup>1</sup>, A. Wendel<sup>1</sup>, A. Kraege<sup>1</sup>, V. Goehre<sup>1</sup>

<sup>1</sup>Heinrich-Heine University, Biology, Düsseldorf, Germany

Smut fungi are a prevalent group of plant-pathogenic fungi that affect agriculturally important cereal crops. In order to establish biotrophic interaction, these pathogens depend on secreted virulence factors called effector proteins. Effectors either promote the virulence by suppressing plant defense responses or alter plant physiology to assist fungal invasion. The infection biology of the grass smut fungi *Ustilago maydis*, *Sporisorium reilianum*, and *Ustilago hordei* has been analyzed intensively to a molecular level, ultimately revealing both common and individual effector repertoires. The Brassicaceae smut fungus *Thecaphora thlaspeos* infects several *Arabidopsis* species as well as model plant *Arabidopsis thaliana* and therefore rises as a novel smut infection system.

In contrast to the rapid infection cycle of *U. maydis*, *T. thlaspeos* establishes a long-lasting biotrophic interaction

with its perennial host plants without causing macroscopic symptoms. Therefore, it would be interesting to explore the unique and conserved effector repertoire of *T. thlaspeos*. Previous studies led to the identification of ~ 50 effector candidates that were grouped into conserved effectors with a homolog in grass smuts fungi, and unique effectors. Among these effector candidates, five have a predicted nuclear localization signal. Transient expression in heterologous systems of the first 3 unique candidates confirmed the predicted nuclear localization. In addition, heterologous delivery by pathogenic bacteria promotes bacterial virulence, suggesting they suppress the plant immune system. We expect that this data might provide a platform for studying, how *T. thlaspeos* effectors enable fungal colonization and disease establishment without causing any macroscopic symptoms.

#### 424-MPP

##### **Communication at the epithelial interface: the transcriptional response of epithelial cells to probiotic and extraintestinal pathogenic *Escherichia coli***

\*H. Klimek<sup>1</sup>, C. Cichon<sup>2</sup>, J. Putze<sup>1</sup>, U. Dobrindt<sup>1</sup>

<sup>1</sup>University of Muenster, Institute of Hygiene, Section of Microbial Genome Plasticity, Münster, Germany

<sup>2</sup>University of Muenster, Center for Molecular Biology of Inflammation, Institute of Infectiology, Münster, Germany

#### Introduction

Epithelial cells in the colon are covered by a mucus layer, which shields the cells from direct contact with bacteria. To study the molecular mechanisms responsible for *E. coli* Nissle 1917's (EcN) well-studied probiotic traits, such as strengthening epithelial barriers and countervailing the colonisation of pathogenic *E. coli*, we use a colonic cell culture model with an adherent mucus layer. To put the mode of action into perspective, we compared the effect of EcN on host cells with the effects of its clonal strains: uropathogenic *E. coli* (UPEC) strain CFT073 and asymptomatic bacteriuria (ABU) *E. coli* isolate 83972, whose genomes exhibit an average nucleotide identity (ANI) of > 99.8 %.

#### Objectives

We intended to establish how the clonal *E. coli* strains interact with the gut epithelial cells that are covered by a mucus layer and to what degree a transcriptional host response to the strains tested would differ.

#### Materials & methods

We cultured HT29-MTX-E12 cells in a transwell system under continuous shaking, to create a semi-wet interface. This triggers the secretion and formation of an adherent mucus layer; the cells polarize, form a three-dimensional architecture and functional tight junctions. The transcriptional host response was analysed via quantitative real time PCR.

#### Results

On the transcriptional level we saw a common innate immune response of the gut epithelial cell line towards *E. coli* strains of different virulence phenotypes, which varied in intensity. *E. coli* Nissle, commonly used as a probiotic supplement, was a strong inducer of leukocyte recruiting factors. This could be confirmed on the protein level.

#### Conclusion

The induction of pro-inflammatory cytokines by EcN was counterintuitive, which raises the question of how probiotic bacteria may protect cells of invasion by pathogenic bacteria. We believe that differential host cell response is an important factor that determines bacterial pathogenicity or probiotic traits.

#### 425-MPP

withdrawn

#### 426-MPP

The low molecular weight protein tyrosine phosphatase PtpB of *Staphylococcus aureus* affects intramacrophage survival and infectivity

\*M. I. Elhawry<sup>1</sup>, L. Pätzold<sup>1</sup>, L. Gannoun-Zaki<sup>2</sup>, V. Molle<sup>2</sup>, S. Becker<sup>1</sup>, M. Bischoff<sup>1</sup>

<sup>1</sup>Saarland University, Institute of Medical Microbiology and Hygiene, Homburg, Germany

<sup>2</sup>Université de Montpellier, Laboratoire de Dynamique des Interactions Membranaires Normales et Pathologiques, Montpellier, France

Secretion of bacterial signaling proteins into host cells to modulate host signaling networks is a common strategy utilized by pathogenic bacteria to subvert the host innate immunity. We reported recently that the  $\sigma^B$ -controlled low molecular weight protein tyrosine phosphatase (LMW-PTPase) PtpA contributes to the infectivity of *S. aureus* by enhancing the intramacrophage survival (Gannoun-Zaki *et al*, J Biol Chem. 293:15569–15580).

Here we show that the second LMW-PTPase produced by *S. aureus*, PtpB, which is constitutively expressed under *in vitro* growth conditions, exerts similar functions. Deletion of *ptpB* in *S. aureus* strain SA564 significantly reduced the capacity of the mutant to withstand the intracellular killing by THP-1 macrophages. When injected into C57BL/6J mice, the SA564  $\Delta$ *ptpB* mutant displayed markedly reduced bacterial loads in liver and kidney tissues in a murine *S. aureus* abscess model when compared to the wild-type. Both phenotypes were fully reverted in a SA564  $\Delta$ *ptpB* derivative that was *cis*-complemented with a functional *ptpB*. Both findings indicate that PtpB exerts a critical role during infection, probably by counteracting the host innate immunity.

#### 427-MPP

PlaD, a novel type IVB secreted effector protein of *Legionella pneumophila*

\*M. Hiller<sup>1</sup>, C. Lang<sup>1</sup>, J. Döllinger<sup>2</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>Robert Koch Institute, Division of Enteropathogenic Bacteria and Legionella, Wernigerode, Germany

<sup>2</sup>Robert Koch-Institut, Division of Proteomics and Spectroscopy, Berlin, Germany

#### Introduction

The facultative intracellular pathogen *Legionella pneumophila* is the causative agent of Legionnaire's disease, a potentially fatal pneumonia. *L. pneumophila* is ubiquitous in aqueous habitats and amoebae are a natural host. However, the pathogen can also colonize lung macrophages and epithelial cells. *L. pneumophila* blocks maturation of the phagosome and thus evades degradation. Instead, the *Legionella* containing vacuole (LCV) is established which allows replication. During infection *L. pneumophila* secretes proteins, among others phospholipases, into the lumen of the LCV and the host cell

cytoplasm via its type II (Lsp) and type IVB (Dot/Icm) secretion systems. At least 15 phospholipases A, which divide into the patatin-like proteins, the PlaB-like proteins and the GDSL hydrolases, are encoded in the genome.

## Objectives

We here focus on the characterization of the phospholipase PlaD which belongs to the family of GDSL hydrolases. We aim to understand the importance and mode of action of PlaD in infection.

## Materials and methods

We investigated the mode of secretion of PlaD by means of Western blotting and translocation assay. Additionally, we determined its binding to various lipids and its interactions with eukaryotic proteins by means of lipid-protein-overlay assays, proximity ligation and pull down assays. Further, we analyzed the localization of PlaD during infection via immunofluorescence.

## Results

We showed that, during infection, PlaD is Dot/Icm-dependently injected into the host cell cytoplasm where it localizes to distinct organelles. Moreover, we demonstrated that PlaD binds to a subset of phosphoinositide species and interacts with a class of regulatory proteins of the host cell. Additionally, our data revealed that the C-terminal half of PlaD is essential for its secretion and phosphoinositide binding but dispensable for interaction with the regulatory proteins.

## Conclusion

Based on its Dot/Icm dependent injection into the host cell cytoplasm, we classify PlaD as a novel type IVB secreted effector protein of *L. pneumophila*. We propose that PlaD is involved in the regulation of host cell signaling cascades during infection.

## 428-MPP

Modulation of HIF1 $\alpha$  by the *C. burnetii* T4SS-effector protein NopA in macrophages

\*I. Hayek<sup>1</sup>, M. Bonazzi<sup>2</sup>, J. Jantsch<sup>3</sup>, A. Lührmann<sup>1</sup>

<sup>1</sup>Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, Mikrobiologisches Institut, Erlangen, Germany

<sup>2</sup>Université de Montpellier, Montpellier, France

<sup>3</sup>Universitätsklinikum Regensburg, Institut für Klinische Mikrobiologie und Hygiene, Regensburg, Germany

**Introduction:** *Coxiella burnetii* is the causative agent of the zoonotic disease Q fever. Apart from acute Q fever, around 2-5% of *C. burnetii* infected humans will develop chronic Q fever, which mainly manifests as endocarditis years after exposure to the pathogen. Usually, humans get infected through the inhalation of contaminated aerosols, often discharged from infected ruminants. Alveolar macrophages are the first line of defence against inhaled *C. burnetii*. Details about how *C. burnetii* are fought by host macrophages and how they escape the immune system and persist for years inside the host are not fully established yet.

**Materials and methods:** Murine bone marrow-derived macrophages (M $\Phi$ ) were infected with different *C. burnetii* clones, under normoxia (N) or hypoxia (H). Samples for western blots, DNA/RNA isolation, and CFU counts were taken. To obtain details for localization and function, the

effector protein NopA was cloned and transfected into host cells.

**Results:** We have shown before that under normoxic conditions, *C. burnetii* replicates in M $\Phi$  while exposure to H impairs *C. burnetii* replication. We proved that this impairment is due to hypoxia-inducible factor 1 $\alpha$  (HIF1 $\alpha$ ) stabilization leading to a cascade of events, which results in the persistence of the pathogen. Interestingly, we noticed that although HIF1 $\alpha$  is stabilized under N 4h after infection, this stabilization diminishes after 24h. This prompted us to investigate whether *C. burnetii* actively modifies the HIF1 $\alpha$  level. Indeed, under H, viable *C. burnetii* reduced HIF1 $\alpha$  stabilization compared to heat-killed or a T4SS-defective mutant, suggesting that *C. burnetii* destabilizes HIF1 $\alpha$  in a T4SS-dependent manner. With the T4SS effector protein NopA we identified one responsible factor for this modification. Moreover, NopA appears to affect HIF1 $\alpha$  stabilization via involving prolyl hydroxylases (PHD). Molecular details of NopA interfering with PHD are still under investigation.

**Conclusion:** Our data suggest that *C. burnetii* attempts to counteract the host defence mechanism through the secretion of the T4SS effector protein NopA, which triggers HIF1 $\alpha$  destabilization.

## 429-MPP

A specific variant of the M-like protein SzM of *Streptococcus equi* subsp. *zooepidemicus* binds IgG, fibrinogen and collagen

\*R. Bergmann<sup>1</sup>, W. Schrödl<sup>1</sup>, C. G. Baums<sup>1</sup>

<sup>1</sup>Institute for Bacteriology and Mycology, Centre for Infectious Diseases, Faculty of Veterinary Medicine, University of Leipzig, Leipzig, Germany

## Introduction

*Streptococcus equi* subsp. *zooepidemicus* (SEZ) is an important pathogen in horses causing pneumonia, arthritis and abortion, but also an invasive zoonotic agent. Its M-like protein SzM is crucial for binding of fibrinogen and for survival in blood. In the related pathogen *Streptococcus pyogenes* distinct M proteins bind collagen via the octapeptide PARF (peptide associated with rheumatic fever) motif. This motif plays a crucial role in the pathogenesis of rheumatic fever in humans. In this study, we describe for the first time an active PARF motif of a variant of the M-like protein SzM of SEZ.

## Aims

We investigated the working hypothesis that the M-like protein SzM\_C31 of SEZ is a virulence factor involved in binding of host proteins like fibrinogen, immunoglobulin and collagen.

## Materials and methods

The recombinantly expressed protein rSzM\_C31 as well as truncated and point-mutated constructs were investigated in Western blots and ELISAs for interactions with IgG, fibrinogen and collagen.

## Results

The M-protein SzM of SEZ strain C31 bound human IgG and IgG of other species via the Fc-fragment. The binding region

was located in the C-term of the protein. Moreover, ELISAs showed binding of equine and human fibrinogen. Sequence analysis of SzM\_C31 identified a PARF motif. Whereas the wt rSzM\_C31 bound collagen, point mutagenesis of the PARF motif resulted in loss of collagen binding. This data is supplemented with phenotypic analysis of the SEZ strain C31 in different assays.

## Conclusion

The SzM\_C31 protein of SEZ is a multi-functional virulence factor, involved in the binding of IgG, fibrinogen and collagen. As the cause of recurrent arthritis in horses is very often not elucidated, we speculate that PARF-motifs in distinct SzM variants of SEZ might be involved in an autoimmune-mediated arthritis in horses.

## 430-MPP

Proteome analysis of *Streptococcus suis* under stress conditions and in host-pathogen interaction

\*D. Dittmar<sup>1</sup>, C. Weiße<sup>2</sup>, B. Jakobczak<sup>3</sup>, P. Hildebrandt<sup>1</sup>, U. Mäder<sup>1</sup>, M. Gesell Salazar<sup>1</sup>, S. Michalik<sup>1</sup>, V. Florian<sup>3</sup>, C. Baums<sup>2</sup>, U. Völker<sup>1</sup>

<sup>1</sup>University Medicine Greifswald, Interfaculty Institute for Genetics and Functional Genomics, Department of Functional Genomics, Greifswald, Germany

<sup>2</sup>University of Leipzig, Faculty of Veterinary Medicine, Institute of Bacteriology and Mycology, Centre for Infectious Diseases, Leipzig, Germany

<sup>3</sup>Ceva Innovation Center GmbH, Geschäftsbereich Tiergesundheit, Dessau-Rosslau, Germany

**Introduction.** *Streptococcus suis*, as a commensal of pigs, can cause invasive infections and is responsible for high economic losses in swine farming worldwide. As an emerging zoonotic agent *S. suis* is able to induce meningitis, arthritis and septicemia. The species comprises 35 serotypes of which serotype 2 is the most prevalent serotype [1], followed by serotypes 9, 7 and 3 in Europe [2]. The mechanisms enabling the switch from commensal to an invasive pathogen are only partially resolved.

**Objectives.** The aim is a comparative profiling of *S. suis* proteome patterns and immunoproteome screening of *S. suis* antigens to reveal the adaptation to host niches and to identify new potential virulence/fitness factors and vaccine candidates.

**Methods.** Proteome analysis of serotype 2, 9 and 7 was carried out with a data-independent acquisition (DIA) mass spectrometry workflow. In addition, *S. suis* specific antibody profiles were investigated using potential immunogenic proteins in a multiplexed suspension bead array.

**Results.** For proteome analysis a spectral library was generated using strain-specific genome sequences and *S. suis* protein extracts from samples grown in different media, at different temperatures and after nutrient or iron limitation. Optimized peptide preparation from low bacteria numbers (106 – 107) [3] from *ex vivo* samples (pig CSF and plasma) and after recovery from infected animals allowed monitoring of around 1100 proteins using the DIA-workflow. The proteome profiling revealed differentially abundant proteins of different cellular pathways. In addition, the antibody response of infected pigs against *S. suis* antigens was quantified by bead-based immunoproteomics. The study revealed proteins, which might be involved in adaptation to the different host niches and constitute potential new virulence factors and vaccine candidates.

**Conclusion.** A combination of immunogenic antigens of different strains specific for the host compartments (CSF and blood plasma) will be selected and screened for suitability as a multicomponent vaccine.

[1] Rungelrath V *et al.*, Virulence 2018

[2] Fittipaldi N *et al.*, Future Microbiol. 2012

[3] Blankenburg S *et al.*, Proteomics 2019

## 431-MPP

**An ABC-transporter is necessary for *Staphylococcus epidermidis* biofilm formation in the presence of human serum**

\*H. Büttner<sup>1</sup>, H. Rohde<sup>1</sup>, A. L. Frank<sup>1</sup>, J. A. Pitann<sup>1</sup>, A. Both<sup>1</sup>, J. Huang<sup>1</sup>

<sup>1</sup>University Medical Center Hamburg-Eppendorf, Clinical Microbiology, Hamburg, Germany

*Staphylococcus epidermidis* is a leading cause of hospital-acquired infections. Invasive disease typically occurs after insertion of implanted foreign material. Key mechanism contributing to *S. epidermidis* pathogenesis of implant-associated infections is biofilm formation on abiotic surfaces. *S. epidermidis* biofilm formation is a highly variable phenotype, single isolates can switch between biofilm-positive and -negative growth depending on environmental conditions. Recently, presence of serum was identified as a factor inducing biofilm formation in otherwise biofilm-negative *S. epidermidis* isolates. We applied comparative genome wide expression analysis of *S. epidermidis* 1585 grown in TSB (no biofilm) or serum (biofilm) and identified several strongly up-regulated genes, indicating significant adaptive responses to *in vivo* like conditions. Two genes (SrE-1 and SrE-2) encoding for transmembrane proteins were chosen for further analysis. Knock-out mutants of SrE-1 and SrE-2 were established in *S. epidermidis* 1585 and *S. epidermidis* 1457. Inactivation did not change colony morphology, growth characteristics in TSB or serum, or antibiotic susceptibility pattern. In both strains, the loss of SrE-1 had no impact on biofilm phenotypes in TSB or serum. Similarly, inactivation of SrE-2 did not change biofilm phenotypes in TSB. However, *S. epidermidis* 1585 and *S. epidermidis* 1457 lost their biofilm positive phenotypes in the presence of serum. In both strains, biofilm phenotypes were rescued by *in trans* expression of SrE-2 under control of an inducible promoter, indicating the genetic linkage between loss of function and biofilm negative phenotypes.

Our work for the first time identified a factor exclusively fostering *S. epidermidis* biofilm formation in the presence of human serum. Obviously, application of assay systems more closely resembling the *in vivo* infection setting is of great importance for an in depth understanding *S. epidermidis* pathogenesis. Work is on the way to decipher the molecular mechanisms linking the loss of a transmembrane transporter and impaired *S. epidermidis* biofilm formation.

## 432-MPP

**Anti-biofilm Effect of Different Titanium Alloys for Implant Materials in Orthopedy with a Focus on *Staphylococcus aureus* and *Staphylococcus epidermidis***

\*L. Wolrab<sup>1</sup>, B. Bödendorfer<sup>1</sup>, B. Lohberger<sup>2</sup>, A. Paulitsch-Fuchs<sup>1</sup>

<sup>1</sup>FH Kärnten, Klagenfurt, Austria

<sup>2</sup>Medical University of Graz, Department of Orthopedics and Trauma Surgery, Graz, Austria

The adhesion of bacteria to artificial joints can cause peri-prosthetic infections (PIs) which are among the most serious complications following joint replacement surgeries. New strategies for inhibiting biofilm development on prostheses involve modifications to the surface and material components. During this project various types of titanium-aluminium-vanadium (TiAl6V4) alloys such as silver-, titanium nitride-, commercially pure titanium-, hydroxyapatite-, tricalcium phosphate- and rough blasted titanium-coatings were tested to assess their anti-biofilm effects. As parameters for biofilm development the amount of proteins and polysaccharides in the formed biofilms were measured and the number of live and dead bacterial cells was used as a measure for the viability. To induce biofilm formation, sterile coin-formed alloy plates were incubated with either *S. aureus* or *S. epidermidis* in 24 well plates with a starting cell density of McFarland 0.5 in lysogeny broth for 48h (37°C and 90 rpm). Biofilms were collected and diluted by scratching them off the surface of the plates and transferred into test-tubes for homogenization in phosphate buffer by vortexing. The protein concentration was determined with the BCA protein assay (Pierce TM, Thermo Scientific) and the polysaccharide concentration with the phenol-sulfuric acid method. Flow cytometry (total cell count) was used as a parameter to assess the effect of the alloys on the microbial growth. Results show that biofilms on rough surfaces (e.g. TiAl6V4-tricalciumphosphate and rough blasted titanium) produced a higher concentration of proteins and polysaccharides whereas on smooth surfaces (e.g. TiAl6V4-TiN) lower concentrations were measured. The cell count of bacteria was higher on rough alloys compared to smooth materials. Moreover, alloys containing titanium-nitride and silver-coating display lower protein and polysaccharide concentrations. These findings confirm that the right choice of implant material could lower the risks of PIs. Future studies should provide more insight into the compatibility and tolerance of the materials by human cells.

#### 433-MPP

##### Anti-biofilm Effects of Different Cobalt-Chrome Alloys for Implant Materials in Orthopedy with a Focus on *Staphylococcus aureus* and *Staphylococcus epidermidis*

B. Bödendorfer<sup>1</sup>, \*L. Wolrab<sup>1</sup>, B. Lohberger<sup>2</sup>, A. Paulitsch-Fuchs<sup>1</sup>

<sup>1</sup>FH Kärnten, Klagenfurt, Austria

<sup>2</sup>Medical University of Graz, Department of Orthopedics and Trauma Surgery, Graz, Austria

Peri-prosthetic infections (PIs), caused by the adhesion of bacteria to artificial joints, are among the most serious complications which can occur following joint replacement surgeries. New attempts to counter biofilm development on prostheses target surface and material components. Accordingly, the aim of this project was to evaluate to which extent various types of cobalt-chrome molybdenum (CoCrMo) alloys, such as titanium nitride (TiN)-, porous-, commercially pure titanium- (cpti), cpti-tricalcium phosphate (TCP)- and highly polished CoCrMo-alloys, have anti-biofilm effects. This was assessed in terms of the amount of proteins and polysaccharides formed by the biofilms as well as the number live and dead bacterial cells. Biofilm formation was induced on sterile coin-formed alloy plates with either *S. aureus* or *S. epidermidis* in 24 well plates (McFarland 0.5, lysogeny broth, 48h at 37°C and 90 rpm). To isolate the biofilm the LB was withdrawn and phosphate puffer was added to each well, the biofilm was scratched off the surface of the material chips and transferred into test-tubes to homogenise it by vortexing. The BCA protein assay (Pierce TM, Thermo Scientific) and the phenol-sulfuric acid method were used to measure protein and polysaccharide concentrations. Furthermore, flow cytometry was used as a

parameter to assess the effect of the alloys on the microbial growth. Data analysis show that rough surfaces (e.g. CoCrMo-cpti) enable biofilms to form higher concentration of proteins and polysaccharides whereas on smooth surfaces (e.g. CoCrMo-TiN) lower concentrations were detected. The bacterial cell number was higher on rough alloys compared to smooth materials. Additionally, CoCrMo with titanium-nitride coating exhibited the lowest results of protein and polysaccharide concentrations. These results substantiate the hypothesis that specific CoCrMo alloys are particularly promising materials to avoid *S. aureus* and *S. epidermidis* biofilm formations in patients with implants. Future studies should focus on the compatibility of the materials with the human body.

#### 434-MPP

##### The scavenger receptor LOX-1 is a novel receptor for *Staphylococcus aureus* WTA on endothelial cells

J. Schade<sup>1</sup>, \*E. Lehmann<sup>1</sup>

<sup>1</sup>Institute for medical microbiology and hygiene, Microbiology and Infection Medicine, Tübingen, Germany

*Staphylococcus aureus*, is an opportunistic pathogen and the most prevalent isolate from patients with endovascular infections. These blood stream infections frequently disseminate into other organs causing subsequent acute or chronic infections. The complex multifactorial process of adherence to the vascular surfaces and subsequent dissemination into the underlying tissue is not yet fully understood. However, adhesion to the endothelial cells is the first crucial step for the onset and progression of an endovascular infection. The important role of protein adhesins in the bacterial binding to the vascular lining has been widely demonstrated. Nevertheless, especially under flow conditions wall teichoic acids (WTAs), major surface components of *S. aureus* were demonstrated to contribute to the binding to human endothelial cells. LOX-1 is a C-type lectin like type-II membrane protein belonging to the family of scavenger receptors which have been implicated in bacterial binding to cellular surfaces. We analysed interaction of *S. aureus* with LOX-1 using adhesion- and ligand-binding-assays. Binding of *S. aureus* to human umbilical vein endothelial cells (HUVECs) was inhibited by preincubation with the natural LOX-1 ligand, OxLDL, or anti-LOX-1 Fab2-fragment. Strains deficient in WTA synthesis ( $\Delta tarO$  mutant) showed a similar reduction in binding to HUVEC. Evidence for a direct interaction of WTA and LOX-1 was provided by *in vitro* interaction experiments in which the parental strain bound a significantly higher proportion of fluorescent-labeled LOX-1 than the  $\Delta tarO$  mutant. Binding experiments with immobilized purified WTA and LOX-1 indicate that secondary modifications of the WTA backbone are crucial for LOX-1 binding. Thus, LOX-1 seems to be an important receptor for WTA mediated adhesion of *S. aureus* during bloodstream infection.

#### 435-MPP

##### Evaluation of inhibitors of *Staphylococcus aureus* virulence in cell-culture based infection models

\*A. Shekhar<sup>1</sup>, U. Bilitewski<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

The ability of *S. aureus* to persist in a variety of host niches and the emergence of antibiotic resistance poses a major health concern. The pathogenesis and host immune evasion of *S. aureus* is attributed to a wide array of virulence factors including cytolytic pore-forming toxins. Alpha-hemolysin forms a homoheptameric pore in host cells facilitating the influx of Ca<sup>2+</sup> and efflux of K<sup>+</sup> and ATP. These processes



lead to cell and tissue damage, and are considered the prime cause of high lethality rates in patients having developed pneumonia caused by *S. aureus*. Inhibition of alpha-hemolysin activity by low-molecular weight chemical compounds can support therapy of these *S. aureus* infections. In a screening campaign, small molecule inhibitors of alpha-hemolysin activity were identified using the recombinant protein and Ca<sup>2+</sup> influx in host cells as an indicator.

In order to evaluate the efficacy of the inhibitors, different infection models were established. They consisted of *S. aureus* in contact with either the lung epithelial A549 cell line or macrophage-like phenotype differentiated THP1 cells as representative host cells. The damaging effect of alpha-hemolysin could be detected with the LDH-release assay or via the determination of necrotic death of host cells. The role of alpha-hemolysin for these cytotoxic effects was determined using the alpha-hemolysin-deletion mutant of *S. aureus* as pathogen and the application of the identified inhibitors. Moreover, Western Blot and ELISA confirmed the presence of alpha-hemolysin in cell-culture supernatants. These studies were complemented by RNA-Seq transcriptome analysis highlighting additional effects of alpha-hemolysin activity and of the inhibitors on host cells.

#### 436-MPP

The transcriptional regulator RpiRb affects peptidoglycan recycling and cell wall thickness in *Staphylococcus aureus*  
R. Gaupp<sup>1</sup>, \*K. Nofal<sup>1</sup>, M. Borisova<sup>2</sup>, H. Peisker<sup>1</sup>, M. Hannig<sup>3</sup>, M. Bischoff<sup>1</sup>, S. Becker<sup>1</sup>, C. Meyer<sup>2</sup>

<sup>1</sup>University of Saarland, Institute of Medical Microbiology and Hygiene, Homburg, Germany

<sup>2</sup>University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany

<sup>3</sup>University of Saarland, Clinic of Operative Dentistry, Periodontology and Preventive Dentistry, Homburg, Germany

The bacterium *Staphylococcus aureus* is one of the most important pathogens that causes a wide variety of diseases in humans. The ability of *S. aureus* to cause a broad range of infections is related to its ability to synthesize virulence factors that facilitate adaptation to specific host niches. The connection between metabolism and virulence regulation became more obvious in recent years and several metabolite-responsive regulators that mediate this linkage have been described. Recently, members of the metabolite-responsive RpiR (ribose phosphate isomerase regulator) protein family have been associated with the utilization of several carbon sources and/or virulence factor biosynthesis in *S. aureus*. Of these regulators, the *rpiRb* gene is part of an operon also containing the *mupG*, *murQ*, and *murP* genes, which are involved in peptidoglycan (PG) recycling. During cell wall turnover, bacteria break down their PG and reutilize the resulting components for the synthesis of new peptidoglycan or as an energy source. Inactivation of the *rpiRb-murQ* operon results in reduced cell wall thickness compared to the isogenic wildtypes. This is interesting because the adaptive resistance towards clinically highly relevant antibiotics like daptomycin (DAP) has been associated with a thickening of the bacterial cell wall. As a result, the reduced PG-thickness of *rpiRb-murQ* mutants has a small effect on the susceptibility to DAP in two DAP-non-susceptible clinical isolates. Furthermore, inactivation of *rpiRb* or the carbon catabolite protein A (*ccpA*) in *S. aureus* altered the transcript levels of the *rpiRb-murQ* operon, demonstrating RpiRb and CcpA as important regulators of peptidoglycan recycling.

#### 437-MPP

Monoclonal antibodies uncover and inhibit the active center of the MprF lipid flippase impairing *S. aureus* virulence and resistance to host defense peptides

C. Slavetinsky<sup>1,2</sup>, \*J. Hauser<sup>1</sup>, C. Ernst<sup>3</sup>, A. Kraus<sup>4</sup>, A. Peschel<sup>1</sup>

<sup>1</sup>Interfaculty Institute of Microbiology and Infection Medicine, Infection Biology, University of Tübingen, Germany, Tübingen, Germany

<sup>2</sup>Department of General Pediatrics, Oncology/Hematology, University Children's Hospital Tübingen, Germany, Tübingen, Germany

<sup>3</sup>Department of Molecular Biology and Center for Computational and Integrative Biology, Massachusetts General Hospital, Boston; Department of Genetics, Harvard Medical School, Boston; The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States of America, Boston, MA, United States

<sup>4</sup>MorphoSys AG, Planegg, Germany

Increasing rates of infections by multi-resistant bacteria prompt both search for novel antibiotics and development of new types of agents to eradicate or weaken invading pathogens. To counteract the increasing therapy resistance of *Staphylococcus aureus*, new anti-infective approaches such as the development of quorum sensing inhibitors, phage therapies, or vaccine have been initiated but are still far from clinical application. The lysinylation of negatively charged phosphatidylglycerol by the multiple peptide resistance factor (MprF) protein represents an important virulence strategy of *S. aureus* to resist cationic antimicrobial host peptides (CAMPs) and antibiotics. MprF is a bifunctional membrane protein consisting of separable domains for lysyl phosphatidylglycerol (LysPG) synthesis and LysPG flipping.

We targeted MprF for the development of a novel antibody-based strategy, to fight *S. aureus* infections. Of several MprF-binding mAbs one (mAB39) was found to sensitize *S. aureus* to CAMPs and the antibiotic daptomycin without affecting LysPG synthesis suggesting that it blocked the LysPG translocation process. mAB39 was found to bind to an MprF loop, which had been localized at the inner surface of the cytoplasmic membrane in earlier studies and by prediction tools. Extensive experimental MprF topology analysis revealed that this loop could be found both, at the outer and inner surface of the membrane suggesting a high structural dynamic, which might be related to the lipid translocation process. Treatment of *S. aureus* with mAB39 resulted in increased killing by CAMP-producing phagocytes and improved bacterial clearance in a mouse skin abscess model. We assume that mAB39 inhibits *S. aureus* by blocking the translocation of Lys-PG and we propose to assess its suitability for the therapy of severe *S. aureus* infection either alone or in combination with cationic antibiotics such as daptomycin.

#### 438-MPP

Unraveling the molecular mechanism determining type III secretion of transmembrane substrates

\*S. Pais<sup>1</sup>, S. Wagner<sup>1</sup>

<sup>1</sup>Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Eberhard Karls University Tübingen, Tübingen, Germany

Type III secretion systems (T3SS) are needle-like molecular machines that allow the transport of proteins across the cell envelope of Gram-negative bacteria, directly into host cell cytosol or membranes to ultimately promote bacterial survival. Among the delivered proteins, named effectors, are those containing transmembrane domains (TMD). Strongly hydrophobic TMD-effectors are prone to be targeted erroneously to the bacterial inner membrane, unless a cognate T3SS chaperone binds the TMD and prevents membrane targeting by the signal recognition particle (SRP). *Salmonella*'s pathogenicity island 2 encodes SscB and SseF

that form a chaperone-effector complex. Previously, it was shown that SscB binds to the chaperone binding domain and the adjacent TMD of SseF. The presence of SscB was required to the correct targeting of SseF to T3SS.

In this work, we aim to elucidate the molecular mechanisms that lead to correct targeting to the T3SS rather than to inner membrane insertion through the Sec-dependent pathway. We are mapping the sites of interaction between SscB and SseF to investigate whether T3SS chaperones recognize special features or only the high hydrophobicity of the substrate's TMD, and if the chaperone is specific to the TMD-effectors. Moreover, we aim to analyze how this interaction is regulated. *sscB* and *sseF* are adjacently located within a single operon, which might be relevant to the regulation of expression and interaction of the two proteins. Currently, we are examining how changes in the order, location, and in the intergenic space of *sscB* and *sseF* can affect the formation of the SscB-SseF complex and SseF secretion. Overall, the presented results will help clarify the events that lead to target discrimination of TMD-effectors.

#### 439-MPP

The use of bacterial chromosomal mutants to study the lipopolysaccharide of *Salmonella*

\*M. Noszka<sup>1</sup>, E. Krzyżewska<sup>2</sup>, J. Rybka<sup>2</sup>

<sup>1</sup>Hirsfeld Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Department of Microbiology, Wrocław, Poland

<sup>2</sup>Hirsfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Department of Immunology and Infectious Diseases, Wrocław, Poland

#### 1. Introduction

*Salmonella* sp. is a Gram-negative rod-shaped, facultative aerobic bacteria, which grows at a temperature from 8 °C to 45 °C and belongs to the *Enterobacteriaceae* family. On the chromosome of *Salmonella* sp. there are fourteen pathogenicity islands, which together with plasmid pSLT encode numerous virulence structures and factors, such as: type III secretion system, Sop protein, H antigen, or lipopolysaccharide (LPS), where the latter molecule builds up the upper layer of the outer membrane cellular envelope of Gram-negative bacteria. Three structural domains can be distinguished in the construction of lipopolysaccharide: lipid A, sugar core and O-specific chain, made up of several repeating polysaccharide subunits, containing from 1 to 8 sugar residues. The O-specific chain shows high variability, thanks to which bacteria use it to avoid phagocytosis and the lytic function of the complement system. The proteins Wzx, Wzz and Wzz are responsible for the biosynthesis of O antigen (OAg), they also have an effect on the control of the O-specific chain length.

#### 2. Aim

The aim of this study was to prepare *Salmonella enterica* subsp. *enterica* serovar Typhimurium PCM 2255 deletion mutants for the *WZZ<sub>lepE</sub>*, *WZZ<sub>ST</sub>* and *wzy* genes, and to analyze their survival in the presence of human serum.

#### 3. Methods

The recombination-mediated genetic engineering (λ Red) technology was used to construct the mutants. For the LPS isolation the Tri-reagent method was used. The lipopolysaccharide analysis was performed using SDS-PAGE electrophoresis. The survival of the mutants was determined by absorbance analysis (λ 600 nm) and measurement of cfu/ml.

#### 4. Results and conclusion

The experiment confirmed the hypothesis - the shorter the O-specific chain, the greater the mortality of bacterial cells upon the action of complement. The shorter OAg chain probably facilitates the absorption of the membrane attack complex (complement system) onto the outer membrane surface, while LPS with a larger number of sugar subunits prevents the direct binding of the MAC to the outer membrane of the bacterial cell, limiting the activity of the complement.

#### 440-MPP

**Role in cell exfoliation of and genomic features associated with α-hemolysin in *Escherichia coli***

\*R. Kolenda<sup>1,2</sup>, U. Frommel<sup>1</sup>, M. Burdukiewicz<sup>3</sup>, M. Noszka<sup>4</sup>, R. Hiemann<sup>1</sup>, J. Weinreich<sup>1</sup>, D. Roggenbuck<sup>1</sup>, P. Schierack<sup>1</sup>

<sup>1</sup>Brandenburg University of Technology Cottbus-Senftenberg, Institute of Biotechnology, Senftenberg, Germany

<sup>2</sup>Wrocław University of Environmental and Life Sciences, Department of Biochemistry and Molecular Biology, Wrocław, Poland

<sup>3</sup>Warsaw University of Technology, Warsaw, Poland

<sup>4</sup>Hirsfeld Institute of Immunology and Experimental Therapy, Department of Microbiology, Wrocław, Poland

#### 1. Introduction

Pathogenic *E. coli* uses various virulence factors to promote fitness and survival during infection. The role and importance of alpha-hemolysin (α-hly) in bacterial colonization or pathogenesis remains not fully elucidated. Possible roles of α-hly in *E. coli* pathogenesis can be exfoliation of epithelial cells to grant access to underlying tissue sites, modulation of host immune response or cell signalling subversion and induction of apoptosis.

#### 2. Objectives

In this study we examined exfoliation of epithelial cells after exposure to hemolysin, investigated the role of α-hly in host cell-specific exfoliation and determined the virulence factor repertoire of hemolytic *E. coli*.

#### 3. Materials & methods

81 hemolytic *E. coli* were tested for cell line exfoliation of intestinal epithelial (LoVo, IPEC-J2) and uroepithelial (5637, PK-15) cells. Genomes of 81 hemolytic and 139 non-hemolytic *E. coli* were sequenced. α-hly sequence was analysed and compared with exfoliation phenotype. Bioinformatic analysis of *E. coli* was conducted with use of Shovill, Prokka, Roary, Scoary, ARIBA, SRST2 and FastTree.

#### 4. Results

Analysis of exfoliation and HlyA sequence revealed presence of HlyA variants responsible for intestinal cell type specific interaction in *E. coli* isolates from pigs. Moreover the single nucleotide polymorphism in *hlyA* and *hlyB* were shown to influence cell line exfoliation irrespectively from cell line origin. Comparison of hemolytic and non-hemolytic *E. coli* revealed presence of different set of genes or gene variants (in case of adhesins) associated with hemolytic *E. coli*. Hemolytic *E. coli* isolates belong to different sequence types and serotypes than non-hemolytic *E. coli*. Genome wide association study was performed and new virulence factors associated with hemolytic and non-hemolytic *E. coli* were found.

## 5. Conclusion

For the first time, exfoliation properties of large set of hemolytic *E. coli* was investigated. We were able to characterize novel variants of  $\alpha$ -hly associated with cell host specificity. Moreover we characterize virulence gene repertoire for  $\alpha$ -hly-bearing *E. coli*.

---

### 441-MPP

Outer membrane protein biogenesis in *Acinetobacter baumannii*: links to virulence and drug resistance

\*K. Leibiger<sup>1</sup>, I. Autenrieth<sup>1</sup>, M. Schütz<sup>1</sup>

<sup>1</sup>Institute of Medical Microbiology and Hygiene, Microbiology, Tübingen, Germany

One of the pathogens ranked with highest priority for development of new therapies are carbapenem-resistant strains of *Acinetobacter baumannii* (*Ab*) [1]. For *Yersinia enterocolitica* (*Ye*) and *Pseudomonas aeruginosa* (*Pa*), our group has previously validated a number of genes (*surA*, *degP*, *bamB*, *bamC*, *skp*) involved in the biogenesis of outer membrane proteins for their potential as novel targets to develop therapeutics that reduce virulence and possibly break resistance. As we had discovered significant differences of the importance of some of our candidate genes in *Ye* and *Pa*, we wanted to determine their relevance also using *Ab*. To this end, we analysed K.O. mutants for *surA*, *degP*, *bamB*, *bamC* and *skp* of a highly virulent multidrug-resistant *Ab* strain (AB5075). A *surA* knockout in *Ye* or a conditional *surA* knockout in *Pa* resulted in reduced serum resistance, enhanced susceptibility to antibiotics and overall reduced virulence of both pathogens [2,3]. Surprisingly, the *surA* knockout mutant of *Ab* only had very weak phenotypes. Our work thus demonstrates the necessity of exhaustive target validation in multiple species. We also started to analyse the redundancy of the periplasmic chaperone network in *Ab*. Surprisingly, and in contrast to *E. coli* it seems that *Ab* possesses an additional bypass of the typical *SurA* and *Skp/DegP* chaperone pathways: we can knock out *surA* and *skp* simultaneously without producing a lethal phenotype (as it is the case in *E. coli* [4]).

[1] Dijkshoorn, L., et al. (2007). *Nat Rev Microbiol* 5(12): 939-951.

[2] Weirich, J., C. Bräutigam, M. Mühlenkamp, M. Franz-Wachtel, B. Macek, I. Meuskens, M. Skurnik, K. Leskinen, E. Bohn, I. Autenrieth and M. Schütz (2017). *Virulence* 8(7): 1170-1188.

[3] Klein, K., M. S. Sonnabend, L. Frank, K. Leibiger, M. Franz-Wachtel, B. Macek, T. Trunk, J. C. Leo, I. B. Autenrieth, M. Schütz and E. Bohn (2019). *Front Microbiol* 10: 100.

[4] Rizzitello AE, Harper JR, Silhavy TJ. *Journal of Bacteriology*. 2001, 183 (23): 6794-6800. 10.1128/JB.183.23.6794-6800.2001.

---

### 442-MPP

Hydrolytic enzymes of *Pseudomonas aeruginosa* as potential virulence factors

\*N. Babić<sup>1</sup>, K. E. Jaeger<sup>1,2</sup>, F. Kovacic<sup>1</sup>

<sup>1</sup>Heinrich Heine University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany

<sup>2</sup>Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences (IBG-1), Jülich, Germany

*Pseudomonas aeruginosa* is an opportunistic pathogen that causes life-threatening infections in immunocompromised patients and is characterized by its high antibiotic resistance. Therefore, the WHO recently classified *P. aeruginosa* as a critical pathogen for research and development of new antibiotics.<sup>1</sup> *P. aeruginosa* produces hydrolases that facilitate degradation of host biomolecules during infections, thereby contributing to virulence through toxic or growth-promoting activities. Among them are several phospholipases and proteases; however, many more hydrolases without known function are thought to be encoded in *P. aeruginosa*.<sup>2</sup>

Our comprehensive sequence homology and 3D structure prediction analyses of *P. aeruginosa* proteins encoded by genes of unknown function revealed 378 putative hydrolases. We aim to experimentally characterize all these hydrolases to identify novel putative virulence factors of *P. aeruginosa*. In a high-throughput approach, we have synthesized by PCR all putative hydrolase genes and cloned them into a newly developed pGUF expression plasmid suitable for heterologous expression in *E. coli* and homologous expression in *P. aeruginosa*. The completion of the expression plasmid library, which presently contains approximately 200 plasmids, is in progress. In first expression experiments using *E. coli* BL21(DE3), eight novel putative hydrolases were expressed as judged by Western blotting. Their unbiased functional characterization using various hydrolase substrates is ongoing.

1. Tacconelli E. et al. (2018) *Lancet Infect Dis*. 18: 318-327.
2. Jaeger, K.-E. and Kovacic, F. (2014) *Methods Mol Biol*. 1149:111-134.

---

### 443-EMP

Multi-purpose *Pseudomonas* B1 strain in downstream process of polyethylene terephthalate (PET) monomers for a circular bioeconomy

\*R. N. C. Utomo<sup>1</sup>, S. Kobbing<sup>1</sup>, T. Narancic<sup>2</sup>, K. O'Connor<sup>2</sup>, N. Wierckx<sup>3</sup>, L. M. Blank<sup>1</sup>, T. Tiso<sup>1</sup>

<sup>1</sup>iAMB- RWTH Aachen, Microbiology, Aachen, Germany

<sup>2</sup>University College Dublin, School of Biomolecular and Biomedical Science, Dublin, Ireland

<sup>3</sup>Forschungszentrum Jülich GmbH, Microbial Catalysis, Jülich, Germany

**1. Introduction:** The global primary production of plastics has been reached 407 million metric tons (MT) in 2015 while polyethylene terephthalate (PET) contributed as much as 33 MT in the market. Surprisingly, 97% of that amount ended as waste and the rest was recycled (1). In the study of *Ideonella sakaiensis* 201-F6 growing on PET, this strain produces two enzymes, namely PETase and MHETase, which first enzyme is capable to hydrolyze PET and followed by the latter enzyme to convert mono (2-hydroxyethyl) terephthalic acid (MHET) into the two monomers terephthalic acid and ethylene glycol (2). In this study, *Pseudomonas* B1 strain, which is isolated from soil rich with brittle plastic waste in a former landfill (Möckern, Leipzig, Germany), was evolved to be able utilize ethylene glycol and terephthalic acid as sole carbon sources.

**2. Objective:** In the context of circular bio-economy, the monomers from depolymerized PET could be utilized by *Pseudomonas* B1 as sole carbon sources to produce valuable product, such as mono-rhamnolipids.

**3. Materials and methods:** Adaptive laboratory evolution was conducted to enable the strain to utilize ethylene glycol.

Subsequently, *tph* operon either by pBT'T vector or by Tn7 transposons was introduced into strain to enable utilizing terephthalic acid.

**4. Results:** The optimum production (concentration and yield) of mono-rhamnolipid from mixed monomers by evolved B1 pBT'T and evolved B1 Tn7 were 0.33 g/L, 0.06 g/g; 0.24 g/L, 0.05 g/g, respectively.

**5. Conclusion:** Integrated strain (evolved B1 Tn7) showed similar result as evolved B1 pBT'T, which is promising strain for industrial as well as environmental application.

#### Reference

1. Geyer R, Jambeck JR, Law KL (2017) Production, use, and fate of all plastics ever made, *Sci. Adv.*, 3, 7.
2. Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda, K (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate), *Science* 351:1196 – 1199.

#### 444-EMP

##### Characterization of Antimicrobial Resistant Genes in Waterborne Bacteria Isolated from Various Water Sources in Abakaliki, Ebonyi State Nigeria.

\*I. Iroha<sup>1,2</sup>, U. U. Ibiam<sup>1,2</sup>, C. Ejikeugwu<sup>1,2</sup>, E. Nwakaeze<sup>1,2</sup>

<sup>1</sup>Ebonyi State University, Abakaliki, Department of Applied Microbiology, Abakaliki, Nigeria

<sup>2</sup>Ebonyi State university Abakaliki, Applied Microbiology, Abakaliki, Nigeria

**Background:** Aquatic ecosystems are recognized reservoir for antibiotic-resistant bacteria and antibiotic resistance genes. The occurrence and spread of antibiotic-resistant bacteria are pressing public health problems that require further clarification. Inadequate water sources for drinking is a serious problem in developing countries like Nigeria especially in rural areas. In this study, the antibiogram and genes responsible for antimicrobial resistance of some important waterborne bacteria were investigated using standard bacteriological and molecular techniques.

**Methods:** A total of 736 water samples comprising boreholes, ponds, rivers, streams and wells which are source of drinking water in Abakaliki Ebonyi State Nigeria were collected and analyzed for the presence of waterborne bacteria pathogens using standard bacteriological and molecular techniques. The antibiogram of isolated bacteria pathogens to conventional antibiotics was determined by disc diffusion method. Screening for the presence of *blaSHV*, class 1 *Integron Ac*, *Int11*, *blaTEM* and *tet* genes in isolated bacteria pathogen was by polymerase chain reaction using specific primers. The clonal relatedness of isolated bacteria was determined using random amplified polymorphic DNA. **Results:** *Aeromonas hydrophila* (n=103), *Escherichia coli* (n=118) and *Vibrio cholerae* (n=87) were the bacteria pathogens isolated from the water samples. They were susceptible to gentamicin (96.12 %), meropenem (94.17 %), ciprofloxacin (89.32 %), imipenem (85.44 %) and chloramphenicol (84.47 %) but resistant to sulphamethoxazole/trimethoprim, kanamycin, azithromycin, streptomycin, cephalothin and cefuroxime. The isolates were found to be positive for antibiotic resistance genes tested namely *blaCTX-M*, *blaSHV*, class 1 *Integron Ac*, *Int11*, *blaTEM* and *tetB*. RAPD of isolated resistance bacteria were resolved into three clusters/groups. **Conclusion:** This study revealed that all the water sources tested were contaminated with waterborne bacteria pathogens which harbors antibiotics

resistant genes and underscores the need for proper water treatment.

**Keywords:** waterborne bacteria, resistant genes, water sources.

#### 445-EMP

Antibiotic resistant bacteria and their resistance genes in biofilm samples isolated from model water distribution systems of Hospitality homes in Benin City, Nigeria.

\*F. O. Ekhaize<sup>1</sup>

<sup>1</sup>University of Benin, Department of Microbiology, Faculty of Life Sciences, Benin City, Nigeria

Water distributed in residential facilities such as Hotels, Inns and Guest houses are generally intended for several purposes like drinking and bathing. It is not sterile, regardless the water treatments been applied. Microbial presence in pipe borne water results in the colonization of the distribution systems infrastructure and biofilm formation. Biofilms are well structured multicellular communities which are buried in a self-produced extra-polymeric substance that functions as an obstacle to antibiotic diffusion. Bacteria in biofilms have some advantage over their free floating counterparts which include protection from disinfectants and ability to resist most antibiotics especially in water piped systems. The aim of this study was to investigate the antibiotic resistance and their resistance genes in biofilms isolated from faucets giving water to end users in hospitality homes. Thirty six biofilm samples were collected from 6 hospitality homes. A total of 108 biofilms bacteria were isolated using spread plate method on R2A medium. Antibiotic susceptibility test was determined using disk diffusion methods. Isolates were characterized using 16S rRNA gene sequencing and three resistance genes; TetA, TetM and ErmB were detected by Polymerase chain reaction. *Alcaligenes faecalis*, *Bacillus cereus*, *Enterobacter* sp., *Lysinibacillus fusiformis*, *Methylobacterium fujisawaense*, *Pseudomonas aeruginosa*, *Providencia vermicola*, and *Serratia liquefaciens*, were isolated. *Alcaligenes* spp. was the most frequently isolated in all Hospitality homes. TetA, resistance gene was more prevalent. It was detected in 49% of biofilm isolates, TetM, 45%, and ErmB, 46% of all biofilm samples. Bacteria isolated from Biofilm were highly resistant to Chloramphenicol (100%) while lowest resistance was reported for Imipenem (1%). In view of the above, there is therefore an urgent need for the Hotel Management to work out modalities in eliminating biofilm bacteria associated with hospitality homes, which could pose a great public health risk due to the presence of multidrug resistant bacteria harboring antibiotic resistant genes

#### 446-EMP

Tiny microbial ecosystems isolated from oil help understanding microbial community assembly

\*L. Voskuhl<sup>1</sup>, V. Brauer<sup>1</sup>, A. Akbari<sup>1</sup>, H. Müller<sup>1</sup>, R. U. Meckenstock<sup>1</sup>

<sup>1</sup>University Duisburg Essen, Biofilm Centre - Aquatic Microbiology, Essen, Germany

The world's largest natural asphalt seep, the Pitch Lake in Trinidad and Tobago, harbors water droplets in microliter size (1-10 µL) originating from the oil reservoir deep below. These water droplets contain diverse and metabolically active microbiomes. In this study we make use of this system to investigate the impact of the anions and cations on microbial community assembly. Ion concentrations were measured with ion chromatography for 46 water droplets

isolated from different sampling sites of the Pitch Lake. The same 46 water droplets were analyzed for their 16S rRNA gene composition by amplicon sequencing and analyzed via mothur pipeline. Multivariate statistics were applied to examine the correlation between sampling site, ion diversity and single OTUs as well as the whole droplet community. For all of the 46 water droplets, more or less different patterns of taxonomic and ion composition were detected.

Based on our measures we can distinguish, if the sampled water droplets originate from the subsurface or if they are an artefact due to sampling or mixture with surface water. We found very similar microbial communities where the inorganic ion concentrations were significantly different but we found also droplets with almost identical ion composition but unequal microbial communities. Our findings show that the ion composition is not determining the microbial community assembly in our system. Only statistically significant correlation between ions and single OTUs was found.

Our findings provide novel insights into these unique and isolated ecosystems and into the role of ion composition on microbial community assembly.

[1] Meckenstock, R.U., *et. al.*, 2014, Water droplets in oil are microhabitats for microbial life. *Science*, 345, 673-676.

---

#### 447-EMP

##### **Seasonal shifts in the Fe-cycling microbial community in sediment from a seasonally hypoxic bay (Eckernförde Bay, SW Baltic Sea)**

\*K. Laufer<sup>1</sup>, C. Bryce<sup>2</sup>, F. Schlotz<sup>1</sup>, V. Nikeleit<sup>2</sup>, G. Gonnella<sup>3</sup>, S. Kurtz<sup>3</sup>, M. Perner<sup>1,3</sup>

<sup>1</sup>GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany

<sup>2</sup>Eberhard Karls Universität Tübingen, Tübingen, Germany

<sup>3</sup>Universität Hamburg, Hamburg, Germany

Fe is an essential nutrient for almost every living organism on Earth. Marine coastal and shelf sediments are known to be an important source of Fe to the water column, where Fe concentrations are typically low and limit primary productivity in large parts of the oceans. Within sediments the fate and characteristics of Fe are controlled and changed by a variety of biotic and abiotic redox-reactions, which together form the benthic biogeochemical iron cycle. The microbial part of this cycle is driven by three physiological types of Fe(II)-oxidizing microorganisms, i.e. microaerophilic, nitrate-reducing and anoxygenic phototrophic Fe(II)-oxidizers, as well as Fe(III)-reducers. The aim of this study was to investigate how changes in bottom water oxygen concentration and sediment redox-stratification impact the abundance, diversity and distribution of the benthic Fe-metabolizing microbial community over sediment depth and over time. To achieve this, we sampled sediment cores for microbial community and geochemical analyses at Boknis Eck, located in the seasonally hypoxic Eckernförde Bay (SW Baltic Sea), at two time points. (i) In autumn, when the oxygen concentration of the bottom water was low and (ii) in the consecutive spring, when the oxygen concentration in the bottom water was higher. The changes in bottom water oxygen concentration were accompanied by changes in sediment geochemistry, e.g. in gradients and content of Fe and H<sub>2</sub>S in the pore water. Based on the presence of 16S rRNA genes, we found that members of all three physiological types of Fe(II)-oxidizers as well as Fe(III)-reducers were present in the sediments at both sampling time points. However, the abundance, diversity and depth-distribution of potential Fe-metabolizers differed between the two sampling time points. We conclude that changes in bottom water oxygen content and sediment geochemistry lead to a change in the composition of the

benthic Fe-metabolizing community and thereby potentially impact the microbial contribution to the benthic biogeochemical iron cycle.

---

#### 448-EMP

##### **Attempts to control microbial community assembly**

\*S. Li<sup>1</sup>, Z. Liu<sup>1</sup>, S. Müller<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research, Environmental Microbiology, Leipzig, Germany

Bacteria grow as community entities in nature. This is the form where they provide their functions driving biochemical processes such as compound production and waste treatment in bio-economy. However, unlike pure strains, knowledge on how microbial communities assemble and vary is still in its infancy. Both stochastic and deterministic processes have been frequently observed in our previous studies. Simple assembly and succession processes of microbial communities or classical niche-based theory cannot explain most of the phenomena observed. The idea of the neutral theory, which regards all organisms in a system equal, could provide an alternative view on processes of community assembly. However, neutral processes might not allow a control of microbial community structures or even functions. Therefore, our aim is to increase deterministic over stochastic processes through manipulated niche selection to strengthen the controllability of microbial community.

One of the strategies which need to be followed will be a testing of environmental disturbance, which selects for certain species combinations and favors deterministic process. By a well-developed workflow based on flow cytometry, the real-time monitoring of the microbial community composition can be realized. Whether the community assembly is predominated by deterministic or stochastic processes can be determined, for instance, by the rank order of abundance distribution of the subcommunities, where a steeper geometric-like slope indicates deterministic processes and a lower lognormal-like slope indicates stochastic processes. This effort to investigate and control the microbial community assembly gives a clue to manageable microbe-driven processes concerning bio-economy.

**Keywords:** microbial community assembly, flow cytometry, deterministic processes, stochastic processes

---

#### 449-EMP

Are bacteria key players in decay and preservation processes?

\*K. Janssen<sup>1</sup>, B. Mähler<sup>2</sup>, G. Bierbaum<sup>1</sup>, J. Rust<sup>2</sup>

<sup>1</sup>Institut für medizinische Mikrobiologie, Immunologie und Parasitologie, Bonn, Germany

<sup>2</sup>Institut für Geowissenschaften und Meteorologie, Bonn, Germany

The fossilization process in aquatic environments is influenced by abiotic and biotic factors. Especially the impact of bacterial activity remains an unexplored territory. In addition to the decay of organisms, bacteria can contribute to the formation of fossils by synthesizing biofilms and biomineralization. There is nothing known about the species that mediate these processes and if they are intrinsic or extrinsic microorganisms. Experiments yielded first insights into the microbial diversity and changes in the composition of bacterial community.

To analyze microbial shifts, the decay of crayfish specimens (*Cambarellus diminutus*) under predefined parameters was observed. Temperature (4°C and 24°C), oxygen status (aerobic and anaerobic) and microbiome of the surrounding medium were altered during decay experiments. For 16S amplicon analysis the V4 variable region of the 16S rRNA gene sequence was amplified and sequenced. All reads with 97 % identity were sorted into different OTUs which were then compared to a curated database of 16S rRNA sequences.

In all test series performed with untreated lake water (4°C and 24°C) Parabacteroides and Clostridium species dominated the decay process. The activity of these genera led to a fast disarticulation of crayfish tissue. In autoclaved lake water fast growing Aeromonas species were highly abundant and mainly at cold conditions showed hardly visible tissue degradation. Some isolated bacterial species contributed to mineralization processes by their metabolic products.

The aim of this project is to shed light on decay and fossilization processes in limnic systems connected to microbial activity. Important for the analysis is the mutual influence of abiotic and biotic factors with special regard to the complexity of the interactions between specific organisms of the microbiome. First experiments showed an alteration of the microbial community composition after the adaptation to changed abiotic parameters. Besides the observation of physical disarticulation, the influence of chemical, physical and biological parameters will be tested to identify the best conditions for mineralization and fossilization of arthropods.

#### 450-EMP

Shifts in microbial community composition and assessment of antibiotic resistance in thermophilic compost from human faeces

\*K. Werner<sup>1,2</sup>, M. Wöhrmann<sup>1</sup>, K. El Said<sup>1</sup>, I. Linkert<sup>1</sup>, T. Hübner<sup>3</sup>, A. Poehlein<sup>4</sup>, R. Daniel<sup>4</sup>, N. Brüggemann<sup>5</sup>, K. Prost<sup>5</sup>, E. Grohmann<sup>1</sup>

<sup>1</sup>Beuth University of Applied Sciences Berlin, Biotechnology, Microbiology, Berlin, Germany

<sup>2</sup>Technical University of Berlin, Environmental Microbiology, Berlin, Germany

<sup>3</sup>Helmholtz Centre for Environmental Research – UFZ, Environmental Microbiology, Leipzig, Germany

<sup>4</sup>Georg-August University of Göttingen, Genomic and Applied Microbiology, Göttingen, Germany

<sup>5</sup>Forschungszentrum Jülich, Institute of Bio- and Geosciences, Agrosphere (IBG-3), Jülich, Germany

In times of tremendous global risks caused by the world-wide climate change the use of sustainable agricultural practices is of primordial importance. In this context, we are investigating the effect of thermophilic composting on the hygienisation of organic waste mainly consisting of human faeces collected from dry toilets in Germany. Our objectives were to gain better knowledge of the microbial community involved in the composting of excreta with a focus on surviving pathogenic species and antibiotic-resistant strains.

Therefore, samples were taken at the beginning and the end of the composting process. We have conducted next-generation sequencing of 16S rRNA gene amplicons to assess the bacterial community shift due to the composting process. Moreover, bacterial isolates were obtained from the mature compost and identified by MALDI-TOF-mass spectrometry. A subset of potential human pathogens will be further characterized regarding their antibiotic susceptibility.

Cultural studies revealed a high percentage of bacteria affiliated to the genera *Pseudomonas*, *Serratia*, *Bacillus* and *Ochrobactrum* in the mature compost as identified by MALDI-TOF mass spectrometry. Approximately 35 % of the isolates were characterized as biosafety level 2 organisms. Elucidation of plasmid content and antimicrobial resistance profiles of selected human pathogenic isolates will help assess the risk of antimicrobial resistance spread during the composting process and give first hints into how to improve the hygienisation potential of the process.

---

#### 451-EMP

*Polaribacter septentrionalilitoris* sp. nov., isolated from the biofilm of a stone from the North Sea

\*S. Choo<sup>1</sup>, J. Wiese<sup>2</sup>, E. Borchert<sup>2</sup>, U. Hentschel<sup>2</sup>

<sup>1</sup>Leibniz Institute for Baltic Sea Research Warnemünde, Department of Biological Oceanography, Rostock, Germany

<sup>2</sup>GEOMAR Helmholtz Center for Ocean Research Kiel, Marine Symbioses, Kiel, Germany

The marine genus *Polaribacter* (class *Flavobacteriaceae*) was first proposed as a psychrophilic/psychrotrophic genus by Gosink et al. (1998). However, many recent members have been deemed mesophilic and environmentally diverse. This study sought to develop a species description of the *Polaribacter* strain ANORD1<sup>T</sup>, and in tandem enhance the current limited knowledge on the general characteristics of this genus.

ANORD1<sup>T</sup> was isolated from the biofilm of a stone along the shore at Nordstrand, Germany and polyphasically characterised. Colony and cell morphological tests were carried out, while physiological and biochemical features were defined via abiotic parameters, metabolic interactions and the biochemical constitution of the strain. The strain's antibiotic resistance profile was also assessed. Importantly, the phylogenetic relationship of ANORD1<sup>T</sup> to other species in the genus was evaluated. To establish a comparison, experiments were simultaneously carried out with the type strains of ANORD1<sup>T</sup>'s next three relatives: *Polaribacter gangjinensis* K17-16<sup>T</sup>, *Polaribacter porphyrae* LNM-20<sup>T</sup> and *Polaribacter reichenbachii* KMM 6386<sup>T</sup> under the same conditions.

Phylogenetic analysis of the 16S rRNA gene sequence showed that ANORD1<sup>T</sup> was most closely related to *P. reichenbachii* KMM 6386<sup>T</sup> (96.7 % 16S rRNA gene sequence similarity), *P. porphyrae* LNM-20<sup>T</sup> (96.9 %) and *P. gangjinensis* K17-16<sup>T</sup> (96.6 %). Recently, a new species (*P. aquimarinus* ZY113<sup>T</sup>) was described (Xu et al, 2019) and is now the closest-related (97.6 %) to ANORD1<sup>T</sup>. Like all *Polaribacter* members, ANORD1<sup>T</sup> is Gram-negative, non-motile, strictly aerobic, MK-6 positive, flexirubin-negative and carotenoid-positive. Optimal growth was observed at 20 – 30 °C, 2 – 7 % salinity and at pH 7 – 10. ANORD1<sup>T</sup>'s major fatty acids differed from the reference strains. ANORD1<sup>T</sup> and the reference strains shared very similar antibiotic profiles, and were all found to be resistant to aminoglycoside-class compounds.

From this polyphasic approach, ANORD1<sup>T</sup> represents a novel species in the genus *Polaribacter*, with the proposed name *Polaribacter septentrionalilitoris* sp. nov.. The type strain is ANORD1<sup>T</sup> (=DSM 110039<sup>T</sup> =NCIMB 15081<sup>T</sup>=MTCC 12685<sup>T</sup>).

---

#### 452-EMP

Interactions of cyanobacteria with predators

\*A. Bauer<sup>1</sup>, E. Kemen<sup>2</sup>, K. Forchhammer<sup>1</sup>

<sup>1</sup>Universität Tübingen, Organismic Interactions, Tübingen, Germany

<sup>2</sup>Universität Tübingen, Microbial Interactions, Tübingen, Germany

Cyanobacteria live as primary producers in ecosystems together with many predators. Their successful propagation in these environments implies efficient defense mechanisms against predation by amoeba, fungi or predatory bacteria. This project aims to look into the recognition of the predator as well as the preparation and execution of the actual defense mechanism. Since the intercellular communication in filamentous cyanobacteria is not limited to adjacent cells but extends to the whole filament, the response of the entire filament during an attack is a main interest of the project. To investigate the defense mechanisms of *Anabaena variabilis*, we started to isolate predatory bacteria from aquatic habitats. From a pond in Tuebingen, we found an unknown predatory bacterium of the phylum Bacteroidetes. A life cell imaging video shows, that it is filamentous and moves by gliding motility (probably driven by chemotaxis) towards its prey, nestles up closely and lyses a cyanobacterial filament within minutes. This finding represents one of the few examples of predatory bacteria feeding on a freshwater cyanobacterium. Recent results show that the spore like cells of *A. variabilis* (akinetes) are resistant to predation of this particular predatory bacterium.

#### 453-EMP

##### The bacterial indoor surface microbiome before and after entry into service of a newly built hospital wing

\*M. Grohmann<sup>1</sup>, A. K. Witte<sup>1</sup>

<sup>1</sup>HTK Hygiene Technologie Kompetenzzentrum, Bamberg, Germany

##### Introduction:

Built indoor environments have shown to be complex and highly dynamic microbial ecosystems. This has, especially in hospital environments, important implications for human health. Moreover, hospitals harbour daily many different patients with high fluctuation and various infections (partially acquired during hospitalisation), are specially cleaned and thus are offering special conditions for the microbiome. The factors that determine the diversity and composition of microbiomes in this environment remain unclear. Objectives: This present study examined the change of the bacterial microbiome before and after opening of a new hospital wing by 16S rRNA gene amplicon sequencing analysis. Further, it aimed to investigate the (long-term) influence of cleaning and disinfection intervention to the microbiome development.

Materials & methods: Swab samples were taken after completed construction work, after final cleaning of the rooms, and at three different time points after occupation and regular operation. Moreover, three rooms were cleaned with disinfectant wipes to monitor if and how the microbiome changes. DNA was extracted, PCR primers were used to amplify the variable regions 3 & 4 of the 16S rRNA and the corresponding libraries were sequenced in a MiSeq system. After raw data quality filtering and processing, community composition and diversity analysis was conducted. Results: Microbiomes of the door handles, floor, sink, remote controls (contact with the patient) and inside of the cabinet (almost no direct contact with the patient) were compared both between each other and between different time points. On samples with direct skin contact (door handles and remote contact), typical dermal bacteria were found. Sink samples offering a different milieu for bacterial growth differentiate from the other samples as expected. Conclusion: The longitudinal study of hospital environmental samples by high-throughput sequencing of bacterial 16S rRNA provides insights into different aspects of the microbial

community composition and can be used for detection of shifts in the microbiome.

#### 454-EMP

##### Glutathione-dependent metabolism in actinobacteria

\*A. C. Lienkamp<sup>1</sup>, T. Heine<sup>2</sup>, D. Tischler<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Microbial Biotechnology, Bochum, Germany

<sup>2</sup>TU Bergakademie Freiberg, Environmental Microbiology, Freiberg, Germany

Actinobacteria are known to utilize mycothiol as main low molecular weight thiol. Analogous to glutathione (GSH), it maintains a reducing environment in the cells and functions as cellular redox buffer. Therefore, occurrence of GSH in actinobacteria is rare. The two verified metabolic roles of GSH in actinobacteria are the degradation of isoprene and the degradation of styrene. Both pathways follow roughly the same scheme, starting with an oxidation of the substrate to its epoxide via a monooxygenase followed by subsequent conversion by a glutathione S-transferase (GST) and a set of dehydrogenases. The final product supplies the central metabolism enabling the use of the respective compound as sole carbon source for the organism. In both cases, some aspects of the pathways still require clarification. Isoprene degradation is known for various microorganisms and is well studied in *Rhodococcus* sp. AD45 whereas GSH-dependent styrene degradation is currently under investigation in *Gordonia rubripertincta* CWB2. Though, it has been described in other microorganisms utilizing an isomerase instead of a GST. Horizontal gene transfer was postulated as origin for these pathways as for many GSH-dependent enzymes. Isoprene is a volatile compound emitted in huge amounts by plants. It is highly reactive and has complex impacts on the climate, health and air quality. Yet, little is known about the general biological cycling of isoprene making its investigation relevant. Additionally, new biotechnological tools could be drawn from the bacterial degradation pathway e.g. new routes for detoxification of chlorinated ethene epoxides. Styrene is a volatile, toxic compound produced in high quantities in industrial processing creating a necessity for its bioremediation and pollution prevention. The central metabolite in bacterial styrene degradation, phenylacetic acid, has industrial value due to its versatile use as precursor for pharmaceuticals, fragrances and flavors. This study presents a comparative analysis of the genomic background and chosen protein sequences including current protein biochemistry research. It aims at an evolutionary understanding of this diverse topic.

#### 455-EMP

##### Daily discharges of antibiotic resistance genes and facultative pathogenic bacteria by wastewater treatment plants with different catchment areas

\*J. Alexander<sup>1</sup>, N. Hembach<sup>1</sup>, T. Schwartz<sup>1</sup>

<sup>1</sup>Karlsruhe Institute of Technology (KIT), Institute of Functional Interfaces (IFI), Department Microbiology / Molecularbiology, Eggenstein-Leopoldshafen, Germany

In total 25 wastewater treatment plants (WWTPs), which contain percentages from livestock and food processing, hospital effluents or municipal wastewater, have been investigated for their daily emission of facultative pathogens and clinically relevant antibiotic resistance genes (ARGs).

The analyzed ARGs included blaTEM, ermB, tetM, sul1 as well as the more clinically relevant ARGs CMY2, CTX-M15, CTX-M32, blaOXA48, blaNDM-1, blaVIM2, KPC-3, vanA, and mcr-1. The facultative pathogenic bacteria included

*Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecium* / *faecalis* as well as *Staphylococcus aureus*. The discharges of ARGs emitted to the aquatic environments averaged about  $4 \times 10^{14}$  cell equivalents (CE) per day. The highest average abundance of ARGs was measured in a WWTP, which treated hospital wastewater. Despite a significantly higher drainage volume, larger sewage treatment plants do not necessarily show an increased emission of ARGs compared to smaller wastewater treatment plants. The emission of facultative pathogenic bacteria in the investigated WWTP effluents were up to 3-log below the average ARG abundances ( $1.34 \times 10^{12}$  CE/day). An important difference between hospital wastewater-affected and non-affected WWTPs was observed in the facultative-pathogenic bacteria. In hospital-influenced WWTPs these bacteria highly correlated with the critical ARGs like bla<sub>NDM-1</sub> (Pearson index: *E. coli* 0.395, *K. pneumoniae* 0.766, *A. baumannii* 0.723, *P. aeruginosa* 0.815, enterococci 0.782) or vanA (Pearson index: enterococci 0.718). Such positive correlations were also observed for other resistance genes against antibiotics of last resorts, but in lower ranges. To avoid a further dissemination of antibiotic resistant bacteria to the aquatic environment, advanced wastewater treatment technologies were investigated. Here, combinations of ozonation with ultrafiltration were found to be most successful to reduce these microbial contaminations below the detection limits. Now, regulations and guidelines are recommended to support the public health protection as recommended by the WHO, UN-Assembly, and G7 summit.

#### 457-EMP

Genomic resolution of a Saccharibacteria-dominated

enrichment culture amended with crude oil/gasoline mixture

\*P. A. Figueroa-Gonzalez<sup>1</sup>, F. Révész<sup>2</sup>, A. Tancsics<sup>2</sup>, A. J. Probst<sup>1</sup>  
<sup>1</sup>University of Duisburg-Essen, Group for Aquatic Microbial Ecology, Institute for Environmental Microbiology and Biotechnology, Essen, Germany

<sup>2</sup>Szent István University, Regional University Center of Excellence in Environmental Industry, Gödöllő, Hungary

#### Introduction

Saccharibacteria represent the best studied phylum of the Candidate Phyla Radiation (CPR), yet little is known about their involvement in hydrocarbon degradation. We recently described the community composition of crude oil/gasoline mix enrichment cultures based on 16S rRNA gene analyses and detected a Saccharibacteria in one of the cultures [1].

#### Objectives

We investigate the strain diversity and metabolism of Saccharibacteria in a crude oil/gasoline enrichment culture.

#### Methods

Duplicates (AER1 and AER2) of aerobic enrichment cultures amended with a mixture of crude oil/gasoline were studied using genome-resolved metagenomics coupled to phylogenomics along with community analyses based on ribosomal protein S3 (rpS3). To study the metabolism at the community level we searched predicted proteins from assembled scaffolds using KofamKOALA HMM profiles. Additionally, selected high-quality genomes were annotated in Genoscoptes" MaGe to elucidate the metabolic potential of Saccharibacteria in the enrichment.

#### Results

Three Saccharibacteria genomes were obtained from AER2, named Sac1, Sac2, and Sac3, but were absent in AER1. Sac1 and Sac2 are the same species (ANI 100%) but depicted different strains distantly related to Sac3 (ANI 73.92%) based on phylogenetic analyses. Sac1 and Sac2 were the most abundant organisms but Sac3 had only an average coverage of eleven. While genomes of Sac1 and Sac2 appeared to have a specialized metabolism (e.g., lactate fermentation), Sac3 revealed a more generic capacity. The adaptation of the community for hydrocarbon degradation was encoded only in other bacterial genomes representing the majority of the rest of the community. Based on relative abundance, a Gammaproteobacteria could be the host of at least one of the Saccharibacteria.

#### Conclusion

The presence of Saccharibacteria in hydrocarbon-degrading enrichment cultures implies an indirect influence of these organisms on the break-down of these compounds by taking up metabolites from hydrocarbon-degrading hosts. The metabolic diversity of Saccharibacteria in this culture suggest a strong adaption to the respective host's metabolism.

[1] Révész, F., et al. Arch Microbiol (2019)

#### 458-EMP

Exploring activity in complex microbial communities using transcriptomic gene expression networks

\*D. Becker<sup>1</sup>, M. Graf<sup>1</sup>, F. Bonk<sup>1</sup>, D. Popp<sup>1</sup>, H. Harms<sup>1</sup>, F. Centler<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany

While metagenomics is a popular technique to analyze the whole genome of microbial communities, metatranscriptomics additionally provides information on the actual metabolic activity (gene expression) of all species present in the community. Besides the analysis of transcribed genetic information of individual species, correlating the activity of genes across species gives rise to gene expression networks (GENs), which can be explored in order to assess activity and detect microbial interactions in the context of anaerobic digestion (AD).

Two continuously stirred tank reactors digesting short chain fatty acids using two feeding regimes (continuously and pulsed) served as the experimental setup. Results of metatranscriptomics (sequenced by the Illumina HiSeq platform) were transferred into GENs using different multivariate clustering approaches.

Both feeding regimes showed similar methane production rates. Metatranscriptomic data revealed a shift of transcriptional activity of the archaea in response to discontinuous feeding compared to continuous feeding. The transcript counts for *Methanosaeta* decreased, whereas *Methanosarcina* counts increased after a feeding pulse. Until the next feeding pulse, the activity level reached to the pre-pulse state. The bacterial community showed only minor differences in transcript counts.

Complex GENs were derived from the metatranscriptomic data. Depending on the applied method, GENs varied. As a proof-of-concept that biologically meaningful results can be obtained from such networks, we found mcrA-subunits to be consistently correlated with each other across all tested methods. Additionally, the propionate degradation pathway could partially be reconstructed.



Metatranscriptomic data showed that AD can be maintained despite discontinuous feeding and that other methanogens become active. The opportunity to reveal activity and interactions within microbial communities using GENs is poorly explored, yet. Our first results indicate that the reconstruction of metabolic pathways based on GENs is in principle possible. To fully assess their utility, however, the derived networks still need to be analyzed in a more systematic manner.

---

#### 459-EMP

##### **Microbial Redox Reactions During Underground Storage of Hydrogen**

A. Dohrmann<sup>1</sup>, \*M. Krüger<sup>1</sup>

<sup>1</sup>BGR, Geomicrobiology, Hannover, Germany

Underground storage of hydrogen (H<sub>2</sub>) could be an alternative or important supplement to store large amounts of surplus electricity gained from renewable energies. However, there is still lack of knowledge about fundamental biogeochemical aspects of underground H<sub>2</sub> storage. The BMBF-funded project H<sub>2</sub>\_ReacT investigates fundamental petrophysical, geochemical and biogeochemical aspects of underground H<sub>2</sub> storage. The work presented here addresses the microbial consumption of H<sub>2</sub> and the involved microorganisms at potential underground storage sites.

Microbial consumption of H<sub>2</sub> is still a major uncertainty factor for underground H<sub>2</sub> storage. Microbial life is widespread in the crust of the earth and geological formations suitable for underground H<sub>2</sub> storage often contain a deep subsurface biosphere. Thus, an underground H<sub>2</sub> storage site needs to be seen as a habitat for microorganisms. Microbial activity at the H<sub>2</sub> storage site might affect the stored H<sub>2</sub> as well as the integrity of the storage site itself. A specific interest is to gain information about microbial activity that might result in a loss of stored H<sub>2</sub> as well as the production of unwanted metabolic products e.g. H<sub>2</sub>S. The importance of specific conditions with relevance for underground H<sub>2</sub> storage i.e. elevated pressure, high temperature and rock material, will be addressed.

Hydrogen consumption by a natural abundant microbial community from a porous rock reservoir fluid was observed. Hydrogen was consumed at different temperature and pressure conditions relevant for H<sub>2</sub> underground storage. Currently effects of several geochemical parameters on microbial H<sub>2</sub> consumption are studied in more detail. Microbial H<sub>2</sub> consumption was shown to be sulfate dependent and led to the formation of sulfide. Furthermore, effects of H<sub>2</sub> consumption on the microbial community composition were studied by high-throughput sequencing of 16S rRNA gene amplicons. Comparative studies identify an increase of sulfate reducing bacteria during microbial H<sub>2</sub> consumption. These results indicate the oxidation of H<sub>2</sub> by sulfate reducing bacteria to be the presumed process in this porous rock reservoir fluid.

---

#### 460-EMP

Microbial hydrocarbon degradation potentials in shoreline and deep-sea sediments around Svalbard and possible ways for their stimulation

S. Bartholomaeus<sup>1</sup>, M. Blumenberg<sup>2</sup>, N. Straaten<sup>1</sup>, S. C. Chen<sup>3</sup>, \*M. Krüger<sup>1</sup>

<sup>1</sup>BGR, Geomicrobiology, Hannover, Germany

<sup>2</sup>BGR, Resource Geochemistry, Hannover, Germany

<sup>3</sup>UFZ, Isotope Biogeochemistry, Leipzig, Germany

The diversity and metabolic potentials of the microbial communities inhabiting sediments in the Northern Barents Sea are largely unknown. Recent reports on natural methane seeps as well as the increase in hydrocarbon exploration activities in the Arctic initiated our studies on the potential of indigenous microbial communities to degrade methane and higher hydrocarbons under in situ pressure and temperature conditions. Furthermore, the seafloor geochemistry in these areas was studied, together with important microbial groups, like methanotrophs, methanogens, metal and sulfate reducers, which may drive seafloor ecosystems in the Northern Barents Sea. A recent focus is on the evaluation of different approaches to stimulate oil degradation by the indigenous microbial populations.

The potential of the indigenous microorganisms to degrade methane and higher hydrocarbons as well as different oils under in situ temperatures and pressures was widespread in surface sediments of the study area. Degradation rates were higher under aerobic than under anaerobic conditions, and decreased with increasing sediment as well as water depths, i.e. increasing pressures. Compared to other sites, like the Gulf of Mexico, rates in shelf surface sediments were in a similar range. However, in greater water depths rates were substantially lower, in accordance with lower cell numbers and the lower in situ temperatures. The addition of different nutrient solutions increased microbial biomass and oil degradation rates. The degradation of the added oils was verified by organic geochemical analyses. Similar activity distribution patterns were found for other metabolic processes, including sulfate, Fe and Mn reduction as well as carbon dioxide and methane production rates. Ongoing molecular biological analyses of original sediments and enrichment cultures show the presence of diverse and varying hydrocarbon-degrading microbial communities.

---

#### 461-EMP

The metabolic relationship of a novel cellulolytic consortium with high potential in lignocellulose degradation

\*R. Rettenmaier<sup>1</sup>, \*Y. Liu<sup>1</sup>, \*S. Vanderhaeghen<sup>1</sup>

<sup>1</sup>Technische Universität München, Lehrstuhl für Mikrobiologie, Freising, Germany

Nature harbours a rich diversity of polysaccharide-degrading bacteria and enzymes but to date the research in this field is mainly concentrated on a few model organisms. In order to find new industrially relevant enzymes for the degradation of plant biomass, it is vital to expand the portfolio of studied organisms and enzymes.

Therefore, cellulolytic organisms were isolated from a mesophilically-operated biogas plant fed with maize silage in previous studies. Interestingly, a mixed isolate, for which 16S rRNA amplicon sequencing indicated novel species or genera, showed high potential in lignocellulose degradation. In contrast to the pure cultures, the consortium metabolizes most of the tested polysaccharides and growth experiments indicated a potential co-evolution stabilized by intertwined metabolic adaptations of the different organisms.

To prove our hypothesis, three pure cultures isolated from the consortium, including a novel member of the family *Lachnospiraceae*, were characterized and their genomes sequenced. In order to clarify the relationships within the consortium, we are performing crossfeeding approaches with poly- and monosaccharides being an integral component of lignocellulose. We are adopting HPLC to analyze the mono-/oligo saccharides released in the supernatant. The secreted enzymes were characterized in the supernatant by secretome analysis via mass spectrometry and by enzyme

assays. Further, we choose enzymes from two gene clusters of one cellulolytic isolate for expression and characterization as this strain has a vast variety of glycoside hydrolase genes for the depolymerization of the major polysaccharides in plant cell wall. The selected enzymes showed high substrate specificities for a synergistic degradation of lignocellulose.

Those experiments will demonstrate the multiple strategies deployed by microbes to efficiently utilize the plant biomass as their diets.

---

#### 462-EMP

Variation in arbuscular mycorrhizal fungal communities associated with lowland rice (*Oryza sativa*) along a gradient of soil salinity and arsenic contamination in Bangladesh

\*S. Parvin<sup>1</sup>, M. Van Geel<sup>1</sup>, T. Yeasmin<sup>2</sup>, B. Lievens<sup>1</sup>, O. Honnay<sup>1</sup>

<sup>1</sup>University of Leuven (KU Leuven), Biology, Leuven, Belgium

<sup>2</sup>University of Rajshahi, Biochemistry and Molecular Biology, Rajshahi, Bangladesh

Rice is an essential food crop that nourishes more than 50% of the world population. In many regions of Bangladesh rice production is constrained by high soil salinity and heavy metal contamination due to irrigation practices. Plants may naturally overcome such stress through mutualistic interactions with arbuscular mycorrhizal fungi (AMF). Yet, little is known regarding the diversity and composition of AMF communities in rice fields with high saline and arsenic concentration. Here we used high throughput Illumina sequencing to characterize AMF communities in rice roots from 45 Bangladeshi rice fields, along a large geographical gradient of soil salinity and arsenic contamination. We obtained 77 operational taxonomic units (OTUs, based on a sequence similarity threshold of 97%) from eight AMF families, and showed that high soil salinity and arsenic concentration are significantly associated with low AMF diversity in rice roots. Soil salinity and arsenic concentration also explained a large part of the variation in AMF community composition, but also soil pH, moisture, organic matter content and plant available soil phosphorus played an important role. Overall, our study showed that even at very high salinity and arsenic levels, some AMF OTUs are present in rice roots. Their potential role in mediating a reduction of rice stress and arsenic uptake remains to be investigated.

---

#### 463-EMP

**High variability of plasmid transfer rates in *Escherichia coli* isolates**

\*K. Flade<sup>1,2</sup>, D. Kneis<sup>3</sup>, S. Heß<sup>1</sup>

<sup>1</sup>TU Dresden, Institute of Microbiology, Dresden, Germany

<sup>2</sup>University, Helsinki, Finland

<sup>3</sup>TU Dresden, Dresden, Germany

Horizontal gene transfer is assumed to play a key role for the spread of antibiotic resistance in aquatic environments. However, it is not known at present to what extent conjugation, transformation and transduction contribute, respectively. In the laboratory, transfer rate constants have been measured for a variety of plasmids, donors and recipients. However, the employed strains typically had a long history in laboratories. Existing data are, therefore, not necessarily representative for real-world environments. Moreover, information on the inter-strain variability of plasmid transfer rates is scarce. Using a high-throughput approach, we studied the transfer of the RP4 plasmid between *Serratia marcescens* (donor) and various *Escherichia coli* recipients (conjugation) and tested the ability to uptake cell-free plasmids by the latter (transformation). The recipients were

isolated from human-borne sewage and river sediments. Comparing the process rate constants of both mechanisms, conjugation was by far more effective. The rate constants of the latter generally followed a log-normal distribution with considerable variance. The conjugation rate constants for good and poor recipients (95 and 5% quantile) differed by more than three orders of magnitude. Specifically, the inter-strain variability of the conjugation rate constant was large in comparison to alterations induced by low-level antibiotic exposure. We did not find evidence for diverging transfer efficiencies between *E. coli* recipients of different origin. On average, strains isolated from river bottom sediments were equally efficient in the uptake of RP4 as isolates extracted from sewage. We conclude that *E. coli* strains persisting in the aquatic environment and those of direct human origin share a similar intrinsic potential for the horizontal transfer of certain plasmids. Our findings underline the possible importance of aquatic systems for the uptake, storage and release of resistance genes. In view of the large inter-strain variability, we propose to work towards probabilistic modeling of the environmental spread of antibiotic resistance.

---

#### 464-EMP

Screening for alkaliphilic cyanide-producing bacteria

\*C. Schuster<sup>1</sup>, J. Sluka<sup>1</sup>, N. Fester<sup>1</sup>, E. Popova<sup>1</sup>, M. Amberger<sup>1</sup>, Y. Tiffert<sup>2</sup>, E. Gabor<sup>2</sup>, \*A. Kletzin<sup>1</sup>

<sup>1</sup>Technische Universität Darmstadt, Mikrobiologie, Darmstadt, Germany

<sup>2</sup>BRAIN AG, Zwingenberg, Germany

**Introduction:** Some microorganisms produce cyanide as secondary metabolite, which can be used for bioleaching of precious metals. They also might provide protective benefits for plant growth, however, the best-known cyanide-producing bacteria *Chromobacterium violaceum* and *P. aeruginosa* are biosafety level-two organisms. Cyanide production is catalyzed by a membrane-associated cyanide-forming glycine dehydrogenase (CGDH) encoded by the *hcn* gene cluster.

**Objectives:** We screened neutral and alkaline soils and pond sediments aiming the isolation of alkaliphilic cyanide-producing bacteria with the biosafety-level one.

**Methods:** Samples collected from different habitats were serially diluted and spread on cyanide-containing solid media (pH 9-12). Cyanide production was assayed by the methemoglobin method. The *hcn* gene cluster of one isolate was expressed in *Escherichia coli*. Modeling of the CGDH was performed with the I-Tasser web server.

**Results:** Among 900 cyanide-resistant colonies, 121 alkalitolerant cyanide-producing microorganisms were isolated from soil samples collected around Darmstadt. The organisms grew at pH 9-10 and were related to the Gammaproteobacteria or Firmicutes, and while Actinobacteria were also identified. In addition, 49 cyanide-producing bacteria were isolated from Austrian soda lakes, most of them belonging to the Firmicutes. Some gammaproteobacterial isolates belonged to biological risk group two so that processing was discontinued. Only a few isolates showed production rates comparable to *C. violaceum*, the most efficient cyanide producer so far.

After expression of the *hcn* gene cluster of one *Pseudomonas* isolate in *E. coli*, cyanide production was in the range of 1-2 mg/L, showing that the CGDH is functional. The three subunits modeled into a heterotrimer showed two central and apparently linked FeS cluster-like arrangements.

**Conclusion:** Numerous bacteria were isolated from different habitats, most of which have not been described as cyanide producers before. The *hcn*-gene clusters in different bacteria do not always have the same gene arrangement but structure predictions are possible and will be discussed.

---

#### 465-EMP

##### **CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> accelerates the iron reduction through phenolic compounds**

\*F. Müller<sup>1,2</sup>, J. Rapp<sup>2</sup>, A. L. Hacker<sup>2</sup>, A. Feith<sup>2</sup>, R. Takors<sup>2</sup>, B. Blombach<sup>1,2</sup>

<sup>1</sup>Technical University of Munich, Microbial Biotechnology, Straubing, Germany

<sup>2</sup>University of Stuttgart, Institute of Biochemical Engineering, Stuttgart, Germany

Iron is essential for almost all kind of life. Despite its great abundance in the Earth's crust, its accessibility for microorganism is often limited because poorly soluble ferric iron (Fe<sup>3+</sup>) is the predominant oxidation state in aerobic environments. The reduction of Fe<sup>3+</sup> is, thus, of central importance to meet the cellular demand of ferrous iron (Fe<sup>2+</sup>). Since previous work had disclosed a link between an increased CO<sub>2</sub> proportion in the inlet air of a bioreactor cultivation and the iron induced transcriptional response of *Corynebacterium glutamicum* (Blombach et al., 2013), this study was intended to characterise the mode of interaction.

We constructed a reporter strain based on the master regulator of iron homeostasis, DtxR, to monitor the intracellular Fe<sup>2+</sup> concentration. To identify the origin of Fe<sup>2+</sup>, iron reduction was addressed in a number of deletion mutants in *C. glutamicum*, enzyme and chemical assays as well as by LC-MS-QTOF analysis of potential reductants.

Our reporter strain indicated a greater activation state of DtxR, when it was cultivated with 20 % (v/v) CO<sub>2</sub> in the inlet air (bioreactor) or when 30 mM NaHCO<sub>3</sub> was supplemented in shaking flasks. We found that CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> did not provide a co-stimulus for DtxR activation nor was there an interaction with the cell physiology. However, HCO<sub>3</sub><sup>-</sup> accelerated the formation of Fe<sup>3+</sup>-protocatechuic acid (PCA) complexes by 46 % and, consequently, enhanced the redox reaction yielding reduced Fe<sup>2+</sup> and oxidised PCA. Other phenolic compounds could replace PCA as reductants in the reaction equivalently. Since the greater intracellular Fe<sup>2+</sup> pool in *C. glutamicum* was apparently caused by the chemical iron reduction and phenolic compounds as well as CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> are wide spread in nature, we think that this abiotic mechanism is of broad significance in other biological as well as geochemical systems.

#### References:

Blombach B., Buchholz J., Busche T., Kalinowski J., Takors R. (2013) Impact of different CO<sub>2</sub>/HCO<sub>3</sub><sup>-</sup> levels on metabolism and regulation in *Corynebacterium glutamicum*. *J. Biotechnol.* 168, 331–340

---

#### 466-EMP

The author has not agreed to a publication.

---

#### 467-EMP

Hidden in soil: Omics approach for the discovery and analysis of natural products from Streptomycetes

\*L. Sagurna<sup>1</sup>, L. S. Kaufmann<sup>1</sup>, T. Busche<sup>2</sup>, C. Rückert<sup>2</sup>, J. Kalinowski<sup>2</sup>, J. Bandow<sup>1</sup>

<sup>1</sup>Ruhr-Universität Bochum, Applied Microbiology, Bochum, Germany

<sup>2</sup>Universität Bielefeld, Center for Biotechnology, Bielefeld, Germany

Natural products are well known to be a great source of compounds for drug discovery. More than 50% of the drugs in use originate from natural products. Hidden in soil, bacteria of the genus *Streptomyces* still have a high potential to produce novel natural products. Although many of their natural products are already known, current omic studies showed that the full biosynthetic potential of *Streptomyces* species has yet to be fully exploited [1].

Here we present a workflow for the discovery and analysis of natural products produced by *Streptomyces* bacteria by combining genomics and metabolomics. As a starting point, to obtain a set of diverse and potentially novel natural product producers, we isolated about 190 bacteria from different soil samples. The growth of the strains was characterized as were the siderophore- and antibiotic-production employing a chrome azurol S (CAS) dye-based assay [2] and cocultivation with gram-positive and gram-negative test strains, respectively. The DNA of the strains was subjected to nanopore and illumina sequencing to identify strains. The assembled genomes were further used to predict biosynthetic gene clusters using the software tool antiSMASH [3] to determine the genetic basis for the biosynthetic potential of the strains.

Thus, we started building a library of isolates from different soil samples and characterized their biosynthetic potential on a genomic level. Future efforts will be directed at the detection of novel natural products using metabolomics [4], their subsequent purification, structure elucidation, and finally their biological and pharmacological characterization.

[1] Maldonado-Carmona et al., *COPHAR* 2019, 48, 1

[2] Schwyn & Neilands, *Anal Biochem* 1987, 160, 47

[3] Blin et al., *Nucleic Acids Res* 2019, 47, 81

[4] Senges et al., *PNAS* 2018, 1152490

---

#### 468-EMP

##### **Improved methods for differentiation of live and dead bacteria in bioaerosol analyses**

\*A. Grünberg<sup>1</sup>, D. C. Pöther<sup>1</sup>, A. Gessner<sup>2</sup>, A. Gärtner<sup>2</sup>, U. Jäckel<sup>1</sup>

<sup>1</sup>Federal Institute for Occupational Safety and Health, Biological Agents, Berlin, Germany

<sup>2</sup>State Office for Nature, Environment and Consumer Protection, Essen, Germany

#### Introduction and Objectives

Bioaerosol exposure can lead to adverse health effects with risk of infection due to alive bacteria. Alive as well as dead microorganisms can cause allergic and toxic effects. Up to now, in bioaerosol analysis culture based methods (CM) are predominantly applied. However, culture independent methods (CIM) have not been tested yet to discriminate alive from dead cells in bioaerosols. Thus, the hazardous potential of bioaerosols is currently hard to assess.

Therefore, the aim of this study was the establishment and comparison of different assays to discriminate alive from dead cells for bioaerosol analyses.

#### Material and Methods

In this study two CIM for live-dead differentiation were established for later bioaerosol analyses. Methodological limitations were determined and results of CIM compared to results of CM. Defined cell counts of heat-inactivated and live bacteria from pure cultures of *Staphylococcus* and *Escherichia* were used for establishing differentiation methods. Quantified ratios of live and dead cells were analysed by (i) live-dead staining prior to fluorescence microscopy and (ii) PMA treatment prior to DNA extraction.

## Results and Conclusion

Heat treatment was found to be the most appropriate method to inactivate cells but maintain cell shape. In both CIM PMA was used for labelling dead cells with defined concentrations for each target species. For both species, no significant differences between expected and determined live-dead-ratios were detected. However, cultivation underestimated the real cell counts by several magnitudes. The results show that the size of cell agglomerates correlates with the underestimation factor for different species.

The newly developed CIM will enable the quantitative differentiation between living and dead bacteria in bioaerosol analyses. Further investigations targeting e.g. the influence of the sampling filters on vitality will lead to a better understanding of the live-dead-ratio in bioaerosols and their potential health risk in different settings.

## 469-EMP

Insights into the microbial community structure and function of a unique terrestrial subsurface ecosystem located in a geologically active rift region

\*D. Lipus<sup>1</sup>, D. Wagner<sup>1,2</sup>, Q. Liu<sup>1</sup>, K. Tinker<sup>3</sup>, D. Gulliver<sup>3</sup>, J. Kallmeyer<sup>1</sup>

<sup>1</sup>GFZ German Research Centre for Geosciences, Geomicrobiology, Potsdam, Germany

<sup>2</sup>University of Potsdam, Institute of Geosciences, Potsdam, Germany

<sup>3</sup>National Energy Technology Laboratory, Geomicrobiology, Pittsburgh, United States

## Question:

With frequent seismic activity, consistently high CO<sub>2</sub> concentrations, and temperatures beyond 100° C, the Eger Rift in Western Bohemia represents a rare subsurface ecosystem and scientifically relevant location to study microbial behavior and bio-geo interactions under extreme conditions. Despite recent advances the role of microbial communities and their metabolic processes in terrestrial subsurface settings remains poorly understood, leaving the question how these microorganisms thrive and survive in such an unique environment unanswered.

## Methods:

To investigate microbial life in this rare environment we collected sediment samples from a core drilled across 240m into an active Mofette as part of the Intercontinental Drilling Program "Drilling the Eger Rift". Applying an innovative DNA extraction approach we were able to recover DNA for genomic investigations from this low biomass environment. Microbial abundance was assessed using qPCR, while 16S rRNA amplicon and metagenomic approaches were utilized to explore microbial community composition and metabolic potential.

## Results:

Microbial abundance ranged between 10<sup>2</sup> to 10<sup>7</sup> 16S rRNA gene copy numbers, and demonstrated microbial life to persist in the terrestrial subsurface below 200 meters. We discovered a novel microbial community dominated by acidophilic and fermentative Proteobacteria and Firmicutes that is driven by pH and has adapted to thrive in the unique subsurface settings. Both qPCR and amplicon sequencing revealed the presence of methanogenic Archaea across core sediments. Reconstruction and annotation of a distinct array of metagenome assembled genomes (MAGs) will allow us to investigate metabolic processes, build metabolic networks, and identify bio-geo interactions potential.

## Discussion:

Our results suggest CO<sub>2</sub> to be a major energy and driving force in this unique ecosystem. Our data also demonstrates that hydrogen liberated from caverns or due to shearing stress in the rocks during seismic events may be utilized by microbes. Altogether, this study advances the current understanding of microbial life in tectonic subsurface systems and provides valuable data for future explorations.

## 470-EMP

Characterization of *Acinetobacter* spp. isolated from input (livestock and avians manure) and output samples of German biogas plants

\*D. Pulami<sup>1</sup>, T. Schauss<sup>1</sup>, S. Mishra<sup>1</sup>, G. Wilharm<sup>2</sup>, J. Bender<sup>2</sup>, J.

Blom<sup>3</sup>, T. Eisenberg<sup>4</sup>, A. Goesmann<sup>3</sup>, P. Kämpfer<sup>1</sup>, S. P. Glaeser<sup>1</sup>

<sup>1</sup>Justus Liebig University Giessen, Institute of applied microbiology, Gießen, Germany

<sup>2</sup>Robert Koch Institute, Wernigerode, Germany

<sup>3</sup>Justus Liebig University Giessen, Institute for Bioinformatics and Systems Biology, Gießen, Germany

<sup>4</sup>Landesbetrieb Hessisches Landeslabor, Giessen, Germany

Studies on the environmental occurrence and distribution of clinically relevant multidrug resistant (MDR), nosocomial and opportunistic *Acinetobacter* spp. are rare. The application of manure as biofertilizer on fields is a critical source for release of MDR bacteria from livestock husbandry into the environment. The aim of this investigation was to study the release of *Acinetobacter* spp. into environment after the anaerobic manure processing in biogas plants (BGPs).

Input and output material of 15 German BGPs collected in 2012 and 2013 were analysed. Abundance of *Acinetobacter* spp. in manure applied to BGPs and the released digestate was quantified by a cultivation-independent approach. *Acinetobacter* spp. isolates cultured by a non-target approach from manure and digestate were characterized in detail phylogenetically by 16S rRNA and *gyrB-rpoB* gene sequencing, *bla*<sub>OXA51</sub>-typing, MALDI-TOF, Multi Locus Sequence Typing (MLST), comparative genomics, and physiological growth properties.

The concentration of *Acinetobacter* spp. 16S rRNA gene copies was in the range of 10<sup>6</sup> to 10<sup>8</sup> per g fresh weight in input material (mixed manure samples) and decreased (partially significantly) to a still quite high concentration in the range of 10<sup>5</sup> to 10<sup>6</sup> per g fresh weight in BGP digestates. Twelve *Acinetobacter* isolates were cultured from input and five from output materials. Four isolates obtained from 3 different input materials and two isolates from 2 digestates were identified as *A. baumannii*. The isolates represented partially known but also new *bla*<sub>OXA51</sub> and ST-types. The strains were able to grow at 42°C (process temperature of BGPs) and survived 14 days of anaerobic incubation. Genome sequence analysis showed the presence of AmpC β-lactamases, several intrinsic resistance efflux and virulence genes in the *A. baumannii* strains. Beside known

intrinsic resistance of this species, all isolates lacked additional resistance against carbapenems, colistin and quinolones.

In conclusion, this study provides initial results of the release of *Acinetobacter* spp. including *A. baumannii* via manure and after the anaerobic treatment of manure in BPGs into the environment.

---

#### 471-EMP

Actinobacterial degradation of 2-hydroxyisobutyric acid

\*T. Rohwerder<sup>1</sup>, M. T. Rohde<sup>2</sup>, N. Jehmlich<sup>3</sup>, J. Purswani<sup>4</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ,

Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Martin-Luther-Universität Halle-Wittenberg, Biophysikalische Chemie, Halle, Germany

<sup>3</sup>Helmholtz Centre for Environmental Research GmbH - UFZ, Molecular Systems Biology, Leipzig, Germany

<sup>4</sup>University of Granada, Institute of Water Research, Granada, Spain

The tertiary branched short-chain 2-hydroxyisobutyric acid has been associated with several metabolic diseases and lysine 2-hydroxyisobutyrylation seems to be a common post-translational modification in proteins. Although the underlying biochemistry is largely unknown, it has already been demonstrated that 2-hydroxyisobutyric acid is degraded by some bacteria, such as the betaproteobacterium *Aquicola tertiaricarbonis* L108 and the bacillus *Kyripidia tusciae* DSM 2912. In these strains, specific ligases and mutases catalyze the CoA activation to 2-hydroxyisobutyryl-CoA and its subsequent isomerization, respectively, to the common metabolite 3-hydroxybutyryl-CoA. In search for alternative routes, we have now investigated the conversion of 2-hydroxyisobutyric acid in the actinobacterium *Actinomycespora chiangmaiensis* DSM 45062. The latter degrades the tertiary branched acid via acetone formation indicating the employment of a lyase reaction instead of the isomerization previously found in strains L108 and DSM 2912. In line with this, strain DSM 45062 does not possess a 2-hydroxyisobutyryl-CoA mutase but a chromosomal gene cluster encoding a 2-hydroxyisobutyrate-CoA ligase and a putative thiamine pyrophosphate-dependent lyase. Comparative proteomics revealed that this ligase-lyase gene cluster and a binuclear iron acetone monooxygenase operon are highly induced when the actinobacterium was grown on 2-hydroxyisobutyric acid. Furthermore, the lyase activity catalyzing the conversion of 2-hydroxyisobutyryl-CoA to formyl-CoA and acetone could be demonstrated in *E. coli* after heterologous expression of the corresponding gene from strain DSM 45062. The presence of the ligase-lyase gene cluster in isoprene-degrading actinobacteria and *Pseudonocardia* strains associated with tropical leafcutter ant species points to a role in degradation of biogenic volatile organic compounds via 2-hydroxyisobutyric acid and related tertiary branched acids.

---

#### 472-EMP

Acetone degradation in *Desulfosarcina cetonica* –

Characterization of recombinant enzymes

S. Kaßner<sup>1</sup>, B. Schink<sup>1</sup>, D. Schleheck<sup>1</sup>, \*J. Frey<sup>1</sup>

<sup>1</sup>University of Konstanz, Biology, Konstanz, Germany

In acetone degradation by aerobic and nitrate-reducing bacteria acetone is activated by carboxylation to acetoacetate which consumes at least two ATP equivalents. For sulfate-reducing bacteria such an expensive activation reaction is hardly feasible, due to their limited energy budget.

Recent studies on the sulfate-reducing bacterium *Desulfococcus biacutus* indicated an involvement of TDP (thiamine diphosphate)- and B<sub>12</sub>-dependent enzymes, leading to acetoacetyl-CoA. In enzyme assays with cell-free extracts of *D. biacutus* an acetone-inducible, B<sub>12</sub>-dependent isomerization of 2-hydroxyisobutyryl-CoA to 3-hydroxybutyryl-CoA, followed by an oxidation to acetoacetyl-CoA by a dehydrogenase was shown. Furthermore, activities of the mutase and the dehydrogenase were identified using recombinant enzymes.

*Desulfosarcina cetonica* is the only other described sulfate-reducing acetone utilizer. The sequenced genome of *D. cetonica* allowed comparative 2D-PAGE and total proteomics analysis leading to the identification of several proteins which are induced during growth with acetone and are potentially involved in acetone degradation. Proteomic data suggested a similar degradation pathway also for butanone and isopropanol.

Several candidate enzymes (a dehydrogenase, a TDP-dependent enzyme and a B<sub>12</sub>-dependent mutase) were successfully cloned and overexpressed in *Escherichia coli*. Purified recombinant enzymes were used for further characterization. The B<sub>12</sub>-dependent mutase linearized 2-hydroxyisobutyryl-CoA to 3-hydroxybutyryl-CoA and a dehydrogenase oxidized 3-hydroxybutyryl-CoA to acetoacetyl-CoA. These two enzymes were further characterized in detail. Nonetheless, the initial activation step (which appears to involve an activated formyl residue) of acetone degradation by *D. cetonica*, is still under research and would represent the last missing piece of this novel biochemical pathway.

---

#### 473-EMP

Identification of novel polyester hydrolases from marine Pseudomonads

\*R. Molitor<sup>1</sup>, A. Bollinger<sup>1</sup>, L. Turkes<sup>1</sup>, S. Thies<sup>1</sup>, K. E. Jaeger<sup>2,3</sup>

<sup>1</sup>Heinrich Heine University Düsseldorf - Institute of Molecular Enzyme Technology, Natural product biosynthesis, Jülich, Germany

<sup>2</sup>Forschungszentrum Jülich GmbH, Institute for Bio- and Geosciences IBG-1, Jülich, Germany

<sup>3</sup>Heinrich Heine University Düsseldorf - Institute for Molecular Enzyme Technology, Jülich, Germany

The extensive use of plastics material has led to a dramatic littering problem in our oceans with patches of garbage as wide as 1.6 million square kilometers. Due to physical erosion, plastic breaks down to plastic particles of only ≤ 5 mm in size. Marine organisms mistaking it for food ingest this so-called micro-plastic as well as macro-plastic, which can lead to congestions and death. Ingested plastic particles may subsequently accumulate through the food web and ultimately end up in humans.

Novel polyester hydrolases may help to fight this urgent problem. To find such enzymes, it seems promising to look for bacteria, which may have naturally adapted to such conditions. As freely floating plastic particles are a perfect medium for colonization, which is a scarce resource in most marine environments, it is reasonable to assume that bacteria have evolved mechanisms to utilize this source of food. Thus, we investigated the potential of marine bacteria to hydrolyze artificial polyesters by using simple screening assays and HPLC after recombinant expression of selected candidate enzymes.

To this end, we developed an agar plate based assay using emulsifiable artificial polyesters like Impranil® DLN and liquid polycaprolactone diol. This fast and easy assay allowed

identifying bacteria of the *Pseudomonas pertucinogena* lineage as a novel source for polyester hydrolases. Purified polyester hydrolases from marine born strains *P. litoralis*, *P. aestusnigri*, *P. pachastrellae*, and *P. oceani* were further analyzed for their capability to hydrolyze polyethylene terephthalate (PET). The latter enzymes indeed degraded PET at 30 °C, however, further improvement of the hydrolytic efficiency is still necessary. In conclusion, we identified novel polyester hydrolases from marine *Pseudomonas* spec. In the future, we intend to screen more marine microorganisms for their polyester hydrolase activity aiming to identify new species producing polyester hydrolases suitable for industrial applications.

#### 474-EMP

Groundwater cable bacteria conserve energy by sulphur disproportionation

\*H. Müller<sup>1</sup>, A. J. Probst<sup>1</sup>, R. U. Meckenstock<sup>1</sup>

<sup>1</sup>University of Duisburg-Essen, Biofilm centre, Essen, Germany

Cable bacteria of the family *Desulfobulbaceae* couple spatially separated sulphide oxidation and oxygen or nitrate reduction by a long-distance electron transfer over centimetre distances. However, the types of energy metabolism of cable bacteria are currently unknown. Here, we report on energy conservation of a highly enriched culture of cable bacteria isolated from an iron-reducing, 1-methylnaphthalene-degrading culture (1MN).

We enriched cable bacteria from culture 1MN with elemental sulphur, either as sole substrate or with additional ferrihydrite or nitrate as electron acceptors. The culture was capable of growing chemo-litho-auto-trophically by sulphur disproportionation when Fe(Cl)<sub>2</sub> was present as sulphide scavenger. Fluorescence *in situ* hybridization (FISH) revealed the presence of *Desulfobulbaceae* as filaments of several hundred micrometres length, but also as single cells or filaments shorter than 50 µm. The filaments showed the characteristic cable bacterial morphology with the continuous ridge pattern over the whole length. From genome-resolved metagenomics, we recovered a nearly complete genome of the enriched cable bacteria. 16S rRNA amplicon sequencing revealed that the cable bacteria enrichment was almost pure with up to 99.8 % abundance of one operational taxonomic unit.

Our results suggest that cable bacteria can conserve energy by substrate-level phosphorylation during sulphur disproportionation. The proposed energy metabolism also explains the energy source of cells that are located in the middle of the cable bacterial filaments where neither sulphide nor oxygen can be measured in environmental samples. These cells probably thrive on sulphur disproportionation which is provided in a cryptic sulfur cycle where sulphide never reaches detectable concentrations.

#### 475-EMP

Dissemination of antibiotic resistances among microbial communities in wastewater and antimicrobial efficacy of low-temperature plasma

\*D. Zühlke<sup>1,2</sup>, D. Schneider<sup>3</sup>, S. Maaß<sup>4</sup>, V. Hahn<sup>5</sup>, J. Kolb<sup>5</sup>, D. Becher<sup>4</sup>, R. Daniel<sup>3</sup>, K. Riedel<sup>1,2</sup>

<sup>1</sup>University of Greifswald, Department of Microbial Physiology and Molecular Biology, Greifswald, Germany

<sup>2</sup>Institute of Marine Biotechnology, Greifswald, Germany

<sup>3</sup>Georg-August-University Göttingen, Department of Genomic and Applied Microbiology, Göttingen, Germany

<sup>4</sup>University of Greifswald, Department of Microbial Proteomics, Greifswald, Germany

<sup>5</sup>Leibniz Institute for Plasma Science and Technology (INP), Greifswald, Germany

Urban wastewater treatment plants (UWTP) are hot spots for the emergence and dissemination of antibiotic-resistant bacteria (ARB). Conventional wastewater treatment is capable of reducing the bacterial load, but may also select for resistant phenotypes. The high bacterial density in UWTP promotes horizontal gene transfer and the presence of antibiotics (ABs), even in sub-lethal concentrations, can select for resistant bacteria. Different technologies, like ozonation, UV-treatment or chlorination, have already been applied for the treatment of wastewater to decrease the amount of ARB that are released into the environment.

Two German UWTP were sampled over a time span of two years. Using a metaproteomics approach, we analyzed the metabolically active microbial community and identified AB-resistance determinants in all stages of the treatment process. Low-temperature plasma was employed on samples from selected wastewater treatment stages and the effect on the microbial community and the presence of resistance-determinants analyzed by metaproteomics. Furthermore, the effect on AB concentrations present in these samples was examined.

Similar taxonomic compositions of the microbial communities in both UWTP were observed with no obvious seasonal influences. Members of the human gut microbiome and bacteria related to infections of the gastro-intestinal/urinary tract that are known to carry AB resistances dominated the untreated sewage community. Treated wastewater contained significantly decreased bacterial numbers but still contains ARB and pathogens. Proteins associated to AB resistances were found at all stages of wastewater treatment. Treatment with low-temperature plasma led to reduction of bacterial numbers. Effects on the amount of resistance-associated proteins and AB residues detected in the wastewater are currently under investigation.

Conventional sewage treatment leads to reduction but not clearance of ARB and potential pathogens. Low-temperature plasma could be a promising treatment technology to eliminate bacteria and ABs and thereby prevent their release into the environment and spreading of AB resistances.

#### 476-EMP

Growth promotion of *Salicornia bigelovii* using rhizosphere-competent polyamine-producing actinobacteria in the United Arab Emirates

\*K. El-Tarabily<sup>1</sup>, B. Mathew<sup>1</sup>, R. Aljneibi<sup>1</sup>, S. AbuQamar<sup>1</sup>

<sup>1</sup>UAE University, Department of Biology, College of Science, Al-Ain, United Arab Emirates

Actinobacteria inhabiting in arid and saline soils can improve plant health. Thirty-five actinobacterial isolates obtained from *Salicornia bigelovii* rhizosphere in the United Arab Emirates, were evaluated for their potential to produce polyamines (PAs) and for their abilities to be rhizosphere-competent. Accordingly, the highly PA-producing *Actinoplanes deccanensis* UAE1 and *Streptomyces euryhalinus* UAE1 were selected. Under greenhouse conditions, application of any of the two isolates significantly ( $P < 0.05$ ) increased the length and weight of shoot and root tissues, photosynthetic pigments and seed yields compared to control plants, suggesting that *A. deccanensis* and *S. euryhalinus* can promote *S. bigelovii* growth. Infestation of soil with these isolates resulted in significant increases in the levels of endogenous PAs and other plant growth regulators (PGRs) including auxins, gibberellic acid and cytokinins, with a

concomitant reduction in abscisic acid in *S. bigelovii* tested tissues. Both *A. deccanensis* and *S. euryhalinus* were, however, incapable of producing significant levels of PGRs *in vitro*. Plant growth promotion (PGP) was strikingly evident in the presence of *S. euryhalinus* than *A. deccanensis*. Thus, this resembling excellence in performance showed the advantage of *S. euryhalinus* as rhizosphere-competent compared to *A. deccanensis* which was not. Our results indicate the importance of rhizosphere competence to be considered for the selection of PGP candidates. This is the first to report the production of PAs by marine actinobacteria; and to demonstrate the potential of PA-producing actinobacteria to enhance the growth of halophytic plants through the increase in the endogenous levels of PAs and other PGRs.

---

#### 477-EMP

Potential to promote the growth of *Salicornia bigelovii* using rhizosphere-competent actinobacteria capable of producing plant growth regulators

\*K. El-Tarabily<sup>1</sup>, S. AbuQamar<sup>1</sup>, B. Mathew<sup>1</sup>, M. AlJneibi<sup>1</sup>, M. Alkhaja<sup>1</sup>

<sup>1</sup>United Arab Emirates University, Biology, College of Science, Al-Ain, United Arab Emirates

This study aimed to isolate and identify rhizosphere-competent plant growth promoting halophilic actinobacteria from *Salicornia bigelovii* rhizosphere and to evaluate their potential as biological inoculants to promote the growth of *Salicornia* in saline soils. Seventy-three actinobacteria obtained from *Salicornia* rhizosphere were initially examined for their abilities to tolerate high concentrations of NaCl. These halophilic isolates were screened for their potential to colonize *Salicornia* roots *in vitro* using a preliminary qualitative indicator root colonization bioassay. The promising 32 isolates which showed moderate to high levels of root colonization were subsequently evaluated for their plant growth promotion traits (phosphorous solubilization, nitrogen fixation, production of siderophores, HCN, ammonia, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase and plant growth regulators (PGRs) including auxins and polyamines. Twelve isolates which exhibited superior abilities to produce PGRs and ACC deaminase were further selected to study their abilities to be rhizosphere competent under a naturally competitive environment using the non-sterilized soil tube assay. Only three isolates which showed outstanding rhizosphere-competent abilities were selected to investigate their effects, individually or in combination, on *Salicornia* seedling growth and on the endogenous levels of auxins, polyamines and ACC in both roots and shoots under greenhouse conditions. While the individual application of each strain resulted in a significant plant growth promotion compared to the control, the combination of the three isolates resulted in superior levels of plant growth promotion. This was also evident from the significant ( $P < 0.05$ ) increases in the levels of photosynthetic pigments, *in planta* auxins and polyamines and the significant ( $P < 0.05$ ) reduction in the endogenous levels of ACC, in roots and shoots compared with control plants. This study is the first report to indicate the potential of halophilic actinobacteria to promote *Salicornia* growth under saline conditions through the production of PGRs and ACC deaminase.

---

#### 478-EMP

Improving phytoremediation of nickel contaminated soil by plant growth-promoting bacteria through the production of the enzyme 1-aminocyclopropane-1-carboxylic acid deaminase in the UAE

\*K. El-Tarabily<sup>1</sup>, S. AbuQamar<sup>1</sup>, L. Alnuaimi<sup>1</sup>, S. Al Zarooni<sup>1</sup>, J. Haj Ali<sup>1</sup>, S. AlDhaheri<sup>1</sup>, A. Taj Elddin<sup>1</sup>, E. Ghazal<sup>1</sup>

<sup>1</sup>United Arab Emirates University, Biology, College of Science, Al-Ain, United Arab Emirates

When heavy metals are present in excess in the soil, they act as toxicants, reducing the success of plant life. Among the metals that are most toxic to plant life are those that displace essential metal ions in biological processes, these include cadmium, zinc, mercury, copper, lead and nickel. Nickel is one of the most abundant heavy metal contaminants of the environment due to its release during mining and smelting practices and it has been the target of many cleanup strategies. High nickel concentrations in the soil have been shown to cause increased ethylene production, inhibit root and shoot development, reduce carbon dioxide fixation and limit sugar translocation. In an effort to gain the advantages provided by bacterial enzyme (1-aminocyclopropane-1-carboxylic acid) (ACC) deaminase in the phytoremediation of heavy metals from the environment, the ability of rhizosphere competent actinobacteria with ACC deaminase activity to promote the growth of corn plants in soils contaminated with nickel was evaluated under greenhouse conditions. To achieve this, 55 different isolates obtained from a corn rhizosphere were selected for their ability to produce ACC deaminase as well as their abilities to tolerate different concentrations of nickel. Out of these isolates, only 18 were able to produce ACC deaminase. Under greenhouse conditions, the application of these actinobacteria increased corn growth in nickel chloride, and nickel nitrate contaminated soils. The application of a mixture of three bacteria in the nickel-contaminated soils significantly enhanced the growth of corn plants compared to the control treatments. Soil inoculation with the mixture of three bacteria in the nickel-contaminated soils significantly enhanced nickel uptake by corn plants from nickel-contaminated soils compared to control treatments which included only nickel without the application of these bacteria. The results suggest that plant growth promoting actinobacteria containing ACC deaminase offer promise as bacterial inoculum for improvement of plant growth particularly under unfavorable environmental conditions such as heavy metals contamination in soils.

---

#### 479-EMP

Structure and Composition of Bacterial Communities in the Rhizosphere of *Hypericum* species

\*Z. Noviana<sup>1</sup>, J. Sikorski<sup>1</sup>, J. Overmann<sup>1</sup>

<sup>1</sup>Leibniz Institute DSMZ, Microbial Ecology and Diversity, Braunschweig, Germany

The plant genus *Hypericum* has recently gained significant importance because some species produce the unique secondary metabolites hypericin and hyperforin. These substances have antidepressant activities and mitigate Alzheimer disease. Despite sparse evidence that some bacterial taxa may increase hypericin production in plant shoot cultures, the role of bacteria in stimulating secondary metabolite production in natural *Hypericum* plants, e.g. via the rhizosphere, has not been explored. As a first step towards an understanding of the role of bacteria-plant interactions, we analyzed the rhizosphere bacterial communities of *H. perforatum*, *H. androsaemum*, and *H. balearicum* by Illumina sequencing of V3 regions of 16S rRNA gene amplicons from both DNA and RNA extracts of rhizosphere and bulk soil samples. We observed not only differences between rhizosphere and bulk soil communities in terms diversity structure and taxonomic composition, but also between RNA-based and DNA-based data indicating differences in transcriptional activities across different taxa. We identified active taxa based on a high rRNA:rDNA ratio

which cannot be explained by stochastic variation in sequence read counts. Our results demonstrate that only 1-2 % of the bacterial rhizosphere communities can be classified as active. Some active taxa are shared across different soil-plant combinations while others are unique for specific combinations. The results warrant further study to determine plant-bacteria interaction between these active taxa and their specific host plant. Our ongoing study involves nine *Hypericum* species to disentangle the effects of plant species from those of soil substrates and includes direct quantification of secondary metabolites.

---

#### 480-EMP

Bed bioelectrochemical reactors: a mosaic of electroactivity

\*A. Kuchenbuch<sup>1</sup>, J. Rodrigo Quejigo<sup>1</sup>, B. Korth<sup>1</sup>, F. Harnisch<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology, Leipzig, Germany

#### Introduction:

Microorganisms having the physical capability of extracellular electron transfer (EET), i.e. to use a solid terminal electron acceptor (TEA), are known as electroactive microorganisms (EAM). In nature, EAM transfer electrons to, e.g., metal ores, whereas in bioelectrochemical systems (BES) electrodes are used as TEA. BES consisting of a granular and porous bed electrode (bed BES) that provides a large surface area for EAM and hence a higher active area for bioelectrochemical reactions are considered as highly promising as the high ratio between active electrode area and reactor volume may pave the way to application<sup>1</sup>.

#### Objectives:

The aim of this study is a characterization of the spatial and temporal heterogeneity of EAM as well as their activity in bed BES. In combination with an analysis of the bioelectrochemical performance, structure-function relationships can be deciphered for fostering engineering of bed BES and for revealing underlying trophic interactions.

#### Material & Methods:

Tubular bed BES with graphite granules as working electrode under potentiostatic control (-0.2 V vs. Ag/AgCl) were operated in batch and continuous flow. Nine minimally invasive sampling sites were integrated to enable electrochemical characterisation of single granules by performing cyclic voltammetry (CV) with the in-house developed eCLAMP2. Additionally, molecular characterisation of the bacterial and methanogenic community was performed.

#### Results:

A high spatial and functional heterogeneity were observed, as eCLAMP CV results show that a high electroactivity was only obtained in proximity to the current collector. In Contrast to the varying electroactivity, the community composition is stable.

#### Conclusion:

Bed BESs are complex 3D-electrochemical environments with a heterogeneous distribution of EAM representing a "mosaic" of electroactivity. The established reactors allowed a representative sampling of the whole bed electrode

providing insights in utilization of the provided electrode surface that will improve future engineering of bed BES.

1 Rodrigo Quejigo, J. et al., 2019 *ChemTexts* 5, art. 4

2 Rodrigo Quejigo, J. et al., 2018 *Electrochem. Commun.* 90, 78 – 82

---

#### 481-EMP

An in vitro characterization of biocontrol mechanism of selected endophytic bacterial strains as biocontrol agent of bacterial wilt disease on chili

\*T. Habazar<sup>1</sup>, Y. Yanti<sup>1</sup>, A. Arneti<sup>1</sup>, N. Muziatul<sup>1</sup>

<sup>1</sup>Andalas University, Plant Protection, Padang, Indonesia

Introduction: Bacterial wilt cause by *Ralstonia syzigii* subsp. *indonesiensis* is the important dangerous disease in chili. Until now this disease has been difficult to control; while the bacteria attack the xylem vessel, they cannot be reached by any bactericide. Biocontrol is potential for controlling this disease. One group of important biocontrol agents is endophytic bacteria (EB). Based on in planta screening method, we have found 10 indigenous endophytic bacterial strains from healthy chili's rhizosphere, which have the ability to control bacterial wilt and to increase growth and yield of chili.

Question: How are the characterers of the direct mechanisms of selected indigenous endophytic bacterial strains as biocontrol agents of *R. syzigii* subsp. *indonesiensis* in vitro?

Methods: The physiological characters of indigenous endophytic bacterial strains as biocontrol agents have been observed, including production of antibiotic, siderophore, HCN, haemolysine, protease, and biosurfactant.

Results: Not all biocontrol tested characters have been produced by indigenous endophytic bacterial strains. Only 3 strains (SLBE1.1BB, SLBE2.1BB and SLBE2.3BB showed the positive on 6 biocontrol characters. All indigenous endophytic bacterial strains produced biosurfactant, the highest viscosity produced by 2 strains (SLBE1.1BB).

Conclusion: Based on these characters, indigenous endophytic bacterial strains consisted of 5 groups.

#### References:

Backman PA and Sikora RA. 2008. Endophytes an emerging tool for biological control. *Biological Control*46:1-3

Edward EJ, King WS, Teck SLC, Jiwan M, Aziz ZFA, Kundat FR, Ahmed OH, Majid AM. 2013. Antagonistic activities of endophytic bacteria against Fusarium wilt of black pepper (*Piper nigrum*). *Int J Agric Biol.* 15(2):291- 296.

Verma P. 2007. Methods for determining bactericidal activity and antimicrobial interactions: Synergy testing, time-kill, curves, and population analysis. In: Schwalbe R, Moore LS & Goodwin AC. (eds.) *Antimicrobial Susceptibility Testing Protocols*, London: CRC Press.

---

#### 482-EMP



Enhancement of growth of seedlings of the mangrove

***Avicennia marina*** by plant growth regulators-producing actinobacteria in the United Arab Emirates

\*K. El-Tarabily<sup>1</sup>, S. AbuQamar<sup>1</sup>, K. Alblooshi<sup>1</sup>, S. Mekky<sup>1</sup>, A. Ali<sup>1</sup>, A. AlMansoori<sup>1</sup>, M. Moustafa<sup>1</sup>

<sup>1</sup>United Arab Emirates University, Biology, College of Science, Al-Ain, United Arab Emirates

This study was aimed at isolating and identifying plant growth promoting actinobacteria from the mangrove *Avicennia marina* rhizosphere and at evaluating their potential as biological inoculants to promote the growth of mangrove seedlings in afforestation programs in the United Arab Emirates (UAE). Nine promising actinobacterial isolates were subsequently evaluated for their ability to produce *in vitro* plant growth regulators (PGRs) (auxins, gibberellic acid, cytokinins and polyamines), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophores, HCN and ammonia. Three isolates were selected based on their superior abilities to produce PGRs and ACC deaminase. Greenhouse experiments were conducted to investigate the effects of these three isolates, individually and in combination on mangrove seedlings growth, photosynthetic gas exchange and endogenous levels of some PGRs in both roots and shoots. The combination of the three isolates resulted in levels of plant growth promotion, superior to those that were exposed to individual strains. This was evident from the significant ( $P < 0.05$ ) increases in the levels of photosynthetic pigments, *in planta* auxins (indole-3-acetic acid and indole-3-pyruvic acid), polyamines (putrescine, spermidine and spermine) and the significant ( $P < 0.05$ ) reduction of the endogenous levels of ACC, the immediate precursor of ethylene, in roots and shoots compared with control plants or plants grown in sediments inoculated with only individual strains. The maximal Rubisco-catalyzed carboxylation velocity ( $V_{c,max}$ ) increased by more than four-fold in plants treated with the mixture of the three actinobacteria over the control. These three isolates are considered to have the potential to perform as plant growth promoters for mangrove production in nutrient impoverished soils in arid coastal areas. This study is the first to demonstrate the potential of indole-3-pyruvic acid, polyamine and ACC deaminase producing PGPR to promote mangrove growth under greenhouse conditions.

#### 483-EMP

##### Metaproteomic Discovery and Characterization of a Novel Lipolytic Enzyme from an Indian hot spring

\*D. Sander<sup>1</sup>, Y. Yu<sup>1</sup>, P. Sukul<sup>1</sup>, T. Mukherjee<sup>2</sup>, S. K. Mukhopadhyay<sup>2</sup>, L. I. Leichert<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Institute of Biochemistry and Pathobiochemistry, Microbial Biochemistry, Bochum, Germany

<sup>2</sup>The University of Burdwan, Department of Microbiology, Burdwan, India

Lipolytic enzymes are produced by animals, plants and microorganisms. With their chemo-, regio- and enantio-specific characteristics, lipolytic enzymes are the preferred sources for novel biocatalysts which can be useful in several industrial applications. They are widely used in the processing of fats and oils, detergents, food processing, paper and cosmetics production and the synthesis of fine chemicals and pharmaceuticals. In this work, we used a new functional metaproteomics approach, first described by Sukul (2017), to screen sediment samples of a hot spring from India for novel lipolytic enzymes. It is the first time this method was used to screen hot spring samples for enzymes which exhibit lipolytic activity. We were able to identify one enzyme which shows activity during the in-gel activity assay with the fluorogenic substrate para-methylumbelliferyl butyrate. DS001 was found in sediment samples from the Indian Bakreshwar hot spring, which has a temperature of

60°C. DS001 showed the highest hydrolysis activity with substrates with shorter chain length substrates (<C8) with the maximum activity observed with p-nitrophenyl butyrate (C4). For substrates with a chain length >C10 significantly less hydrolysis activity was observed. Preference for short chain acyl groups is characteristic for esterases, suggesting that DS001 is an esterase. Because there is an increasing demand for esterases which can be used in the synthesis of enantiomerically pure compounds, we determined the enantioselectivity of DS001 with 4-methylumbelliferone-(R)-2-ibuprofen and 4-methylumbelliferone-(S)-2-ibuprofen. DS001 shows a modest preference for 4-methylumbelliferone-(R)-2-ibuprofen. DS001 shows a temperature optimum at 55°C and more than 50% activity in a range between pH 8.0 and pH 9.5 where it has its optimum. It also exhibits a high tolerance to organic solvents in a concentration of 1% (v/v). In fact, it still shows 80% activity in the presence of 1% acetone. 1% of methanol increases the activity of DS001 to 140% in comparison to the optimum conditions without solvent. In the presence of 10% methanol, DMSO or isopropanol it still shows around 50% activity.

#### 484-EMP

##### Spatial-temporal dynamics of microbial communities in floodplain sediments

\*L. Cramaro<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München GmbH, Institute of Groundwater Ecology (IGOE), Neuherberg, Germany

Floodplain soils are considered hotspots of biogeochemical activities driven by strong seasonal dynamics of precipitation, soil water saturation and redox conditions. However, the interlinkage of abiotic conditions and biotic processes governing carbon, nutrients and contaminants (e.g. pesticides) turnover are poorly understood. In my work, integrated in the DFG CRC 1253 CAMPOS project, I particularly aim to evaluate the link between soil moisture and redox dynamics with changes in the composition and functional status of the soil microbial communities. We hypothesize that seasonal hydrological events cause redox dynamics that translate into dynamics in microbial key processes, while the composition of communities stay stable. Herbicide applications, on the other hand, are assumed to impact composition and function.

Site of investigations is the Ammer valley floodplain located in the area between Tübingen and Pfäffingen (Baden Württemberg), characterized by agriculturally impacted alluvial and colluvial soils. A monthly sampling took place from March 2018 until August 2019 at two different locations, i.e. a barley planted field occasionally treated with herbicides (including glyphosate) and a manure fertilized meadow. Measures included the dynamics of key environmental parameters such as precipitation, recharge, soil water content, pore water chemistry and redox potential. Soil microbial communities were analyzed in terms of activity and bacterial fingerprints.

Porewater isotopic signatures indicate a significant recharge in spring and preferential infiltration through macropores into the fractured loamy soils. Soils of the two investigation sites show similar redox potentials with depth. Moreover, first data show seasonal changes in the number of microbes in depth until 10 cm, especially in the field. Increased microbial activities were observed always in spring (April to June) with higher values but less dynamics in meadow soil. Currently samples are further analyzed by means of deep amplicon sequencing and quantification of functional genes related to nitrogen and herbicide turnover.

---

#### 485-EMP

##### **Application of Whole Cell Biosensors for Identifying *Streptomyces* spp. as Sources for Novel Polyene Antibiotics**

\*D. Hartmann<sup>1</sup>, F. Dürr<sup>1</sup>, A. R. Guarinos<sup>1</sup>, T. Mascher<sup>1</sup>

<sup>1</sup>TU Dresden, Allgemeine Mikrobiologie, Dresden, Germany

Antibiotic resistant microbes are on the rise and pose one of the biggest public health challenges of our times. In times of so-called superbugs, which are resistant even against the antibiotics of last resort, new antimicrobial compounds are direly needed to combat these germs. But the progress in developing new drugs is slow and the pipelines are almost dried out. Even after decades of successful exploitation, filamentous, soil bacteria from the genus *Streptomyces* spp. are still very important potential sources for new antibiotics because of the richness and diversity of their secondary metabolism. But novel approaches are required to tap this rich source, in order to identify new compounds. A promising – and underappreciated – approach is the use of compound- or target-specific whole cell biosensors; bacteria that give an easily quantifiable and specific reporter output in response to exposure to a specific chemical compound. Here, we screened a collection of *Streptomyces* soil isolates for induction of a novel amphotericin B-specific biosensor. One isolate that gave rise to a strong and specific signal was chosen for subsequent in-depth analyses. Multi-locus sequence typing and whole genome sequencing was applied and a draft genome sequence for this new isolate of *Streptomyces* sp. #4/1 was obtained. Comprehensive genome mining was performed for identifying antibiotic biosynthesis clusters and three potential candidate clusters that might encode polyene biosynthesis pathways were identified. After optimization of the growth conditions, active cell extracts were generated and further analyzed for isolation and characterization of the corresponding compounds. Our approach demonstrates the power and efficiency of combining whole cell biosensors with genome mining for identifying new antibiotics.

---

#### 486-EMP

##### **Biotransformation of organic micropollutants di-*n*-butyl phthalate (DBP) and diethyl phthalate (DEP) by fungi of different ecophysiological groups**

\*L. Carstens<sup>1,2</sup>, A. R. Cowan<sup>2,3</sup>, B. Seiwert<sup>4</sup>, D. Schlosser<sup>2</sup>

<sup>1</sup>Universität Duisburg Essen, Biofilm Centre, Aquatische Mikrobiologie, Essen, Germany

<sup>2</sup>Helmholtz-Zentrum für Umweltforschung GmbH, Umweltmikrobiologie, Leipzig, Germany

<sup>3</sup>University of Glasgow, Glasgow, United Kingdom

<sup>4</sup>Helmholtz-Zentrum für Umweltforschung GmbH, Analytische Chemie, Leipzig, Germany

Phthalate esters (PEs, phthalates) are environmentally ubiquitous micropollutants arising from their extensive use as plasticizers and additives in diverse consumer products. Considerable concerns relate to their reported xenoestrogenicity. Consequently, the potential towards microbial-based attenuation of PE concentrations in different compartments of the environment is of interest to combat harmful downstream effects. Fungal PE catabolism has received less attention than that by bacteria as of yet, and in particular fungi beyond common terrestrial species have been less intensively investigated in this respect. We have compared the biocatalytic and biosorptive removal rates of DBP and DEP, chosen as environmentally prominent PE representatives with different structures and hydrophobicities, by marine- and freshwater-derived fungal strains as well as fungi associated with various terrestrial habitats. Partial pathways for DBP metabolism by

ecophysiologically diverse asco- and basidiomycete strains could be proposed with the help of UPLC-QTOF-MS analysis. Individual patterns of the major biochemical reaction steps contributing to DBP metabolism were observed for the investigated strains. The involved reactions include initial cytochrome P450-dependent monohydroxylations of DBP with subsequent further oxidation of related metabolites, de-esterification via either hydrolytic cleavage or cytochrome P450-dependent oxidative O-dealkylation, transesterification, and demethylation steps, finally yielding phthalic acid as a central intermediate in all pathways.

---

#### 487-EMP

##### **Seasonal Differences of Coliform Bacteria in Drinking Water Reservoirs**

\*C. Reitter<sup>1</sup>, A. Hesselschwerdt<sup>1</sup>, H. Petzoldt<sup>2</sup>, J. Gescher<sup>3</sup>, M. Hügler<sup>1</sup>

<sup>1</sup>TZW: DVGW-Technologiezentrum Wasser, Microbiology and Molecular Biology, Karlsruhe, Germany

<sup>2</sup>TZW: DVGW-Technologiezentrum Wasser, Dresden, Germany

<sup>3</sup>Karlsruhe Institute of Technology, Applied Biology, Karlsruhe, Germany

**Introduction:** Microbial water quality is examined using bacterial indicators such as coliform bacteria, *Escherichia coli* and enterococci. Their detection indicates a possible fecal contamination. For coliform bacteria this concept has been relativized as they also occur in the environment. However, their hygienic relevance remains unclear. In recent years this issue has become more important due to mass proliferation of coliform bacteria in drinking water reservoirs throughout Germany which challenges the drinking water treatment.

**Objectives & Methods:** The aim of our study was to analyze and identify microbial indicator bacteria that naturally occur in drinking water reservoirs in Germany and to find seasonal differences especially during mass proliferation events. Growth experiments were conducted with these strains to find special adaptations to oligotrophic waters. Furthermore the microbial community was examined and microbial source tracking markers were tested.

**Results:** Our monitoring study demonstrates seasonal differences in the microbiology of drinking water reservoirs. During summer the maximum density of coliform bacteria reached values above 10<sup>3</sup> MPN/100 ml with only a few strains of coliform bacteria being dominant. These strains belong to the genera *Lelliottia* and *Enterobacter* and closely related strains were found in different reservoirs all over Germany. Growth experiments have shown that these strains have different growth potential according to the water and conditions being used. They were capable to grow in raw water but not in treated drinking water. Fecal indicators such as *E. coli* and enterococci were rarely detected. In contrast to the water body of the reservoir the inflows showed no significant seasonal differences.

**Conclusion:** This and the fact that no microbial source tracking markers could be detected leads to the assumption that the proliferation is an autochthonic process within the water column. Due to climate change, leading to higher water temperatures and fluctuating water levels, such mass proliferations of coliform bacteria are expected to occur more frequently in drinking water reservoirs in the near future.

---

#### 488-EMP

## Temporal and spatial variation of lake microbiomes in the Pyrenees

\*J. Laufer<sup>1</sup>, R. Kallies<sup>1</sup>, D. S. Schmeller<sup>2</sup>, A. Loyau<sup>3,2</sup>, A. Chatzinotas<sup>1,4</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research GmbH - UFZ, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>ECOLAB, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France

<sup>3</sup>Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Department of Experimental Limnology, Stechlin, Germany

<sup>4</sup>German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

Aquatic mountain ecosystems are exposed to increasingly harsh environmental conditions due to climate change, including extreme weather events and a shortened and increasingly variable growth season. Freshwater microorganisms, which play a crucial role in nutrient cycling, need to adapt to fluctuations of abiotic conditions to maintain lake functioning. However, temporal and spatial variations in microbial community composition are rarely investigated over several years, which limits our understanding of their variability with respect to environmental factors.

Here, we disentangle which environmental parameters (e.g. geographical, chemical and hydrogeological parameters) shape the water microbiome composition for bacteria and micro-eukaryotes, including protists and fungi. We hypothesize that community composition (1) reflects the effects of stable, lake specific characteristics as well as the effect of factors that fluctuate according to seasonal cycles and (2) that co-occurrence networks within and across these trophic levels will reveal inter-dependencies between different microbial groups.

We sampled 28 Pyrenean lakes with three seasonal sampling campaigns in 2016 to 2018, yielding more than 700 freshwater samples. The DNA of these samples was extracted, amplified and highly variable regions of the 16S rRNA (bacteria) and 18S rRNA (micro-eukaryotes) encoding genes were sequenced using next generation sequencing technologies (Illumina MiSeq). Biogeochemical data and hydrogeological characteristics were obtained for each of the investigated lakes.

In total, we found 26.915 bacterial and 12.040 eukaryotic ASVs. Proteobacteria and Bacteroidetes dominated among bacteria, while members affiliated to SAR (mostly Dinoflagellata, Ochrophyta and Ciliophora) and Ophisthokonta (mostly Fungi) dominated eukaryotes. First results show a high fluctuation of rare species within both bacterial and protistan communities. The abundance pattern of individual taxa was highly variable between years and seasons, leading to the conclusion that sampling of only a single time point misses the full picture of community diversity and composition in aquatic mountain ecosystems.

## 489-EMP

withdrawn

## 490-EMP

Assessing variability of DADA2 pipeline on different parameters and databases

\*A. Bartholomaeus<sup>1</sup>, S. Liebner<sup>1</sup>, D. Wagner<sup>2,1</sup>

<sup>1</sup>Helmholtz Centre Potsdam, GFZ German Research Centre for Geosciences, Section 3.7 Microbiology, Potsdam, Germany

<sup>2</sup>University of Potsdam, Institute of Geosciences, Potsdam, Germany

DADA2 has become one of the standard tools to analyse 16S rRNA amplicon data and uncover microbial community

compositions. However, the results obtained from the pipeline can vary strongly based on used processing options and parameters. In addition, there are different taxonomic database and versions that are frequently updated, extended and reordered. As a result microbial communities detected from the same samples but by different approaches and at different times are dissimilar. The geomicrobiology section at the GFZ is working in extreme environments, such as the Atacama Desert, permafrost environments and the deep biosphere, which complicates the analysis further due to low biomass in these environments and high fraction of potential unknown microorganisms. We examined the variability of detected microbial communities from different extreme environments leading to insights into reproducibility of the results and direct rules of best practice for application of parameters and databases.

## 491-EMP

### Targeted cultivation of Planctomycetes

\*M. Salbreiter<sup>1</sup>, \*M. Waqqas<sup>1</sup>, N. Kallscheuer<sup>2</sup>, S. Wiegand<sup>2</sup>, A. Heuer<sup>3</sup>, C. Boedeker<sup>3</sup>, P. Rast<sup>3</sup>, R. W. Müller<sup>4</sup>, F. Brümmer<sup>4</sup>, M. S. M. Jetten<sup>2</sup>, M. Rohde<sup>5</sup>, M. Jogler<sup>1,2</sup>, C. Jogler<sup>1,2</sup>

<sup>1</sup>Friedrich-Schiller-University, Jena, Germany

<sup>2</sup>Radboud University, Nijmegen, Netherlands

<sup>3</sup>Leibniz Institute DSMZ, Braunschweig, Germany

<sup>4</sup>Institute of Biomaterials and biomolecular Systems, Stuttgart, Germany

<sup>5</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

Planctomycetes are maverick bacteria with an exceptional cell biology that are environmentally important in global carbon and nitrogen cycles. They dwell in all sorts of habitats, in particular in the association with Eukaryotes. Despite their slow growth, Planctomycetes can dominate the bacterial community of such habitats employing small molecules that might be of biotechnological interest.

Unfortunately, only few planctomycetal axenic cultures exist, limiting the discovery process of small molecules from planctomycetal origin. To overcome this limitation, we employed a targeted cultivation approach focusing on marine surfaces. Kelp macroalgae and *Posidonia* seagrass leaves are known to be hotspots of planctomycetal occurrence. Up to 80% of the bacterial community on the surface of these eukaryotes can be planctomycetes. Thus, we sampled giant kelp (*Macrocystis pyrifera*) at Monterey bay, CA, USA, *Posidonia* seagrass leaves from Panarea, Italy and macroalgae from Mallorca, Spain. As abiotic control, we used polyethylene particles that were incubated in the Baltic Sea at Heiligendamm, Germany for 2 weeks. In total, we obtained six novel strains that we characterized in detail to allow a species description. Phylogenetically, the strains form two novel genera that we suggest to name *Symmachiella* and *Rosistilla*. The genera *Symmachiella* and *Rosistilla* have only 89% and 92% 16S rRNA sequence identity to their next planctomycetal relatives. Three strains form the novel genus *Symmachiella*. Strain Pan258 was isolated from *Posidonia* leaves collected close to a subsea volcanic gas escape at Panarea Italy. Strain Mal52 was obtained from algae collected at the island Mallorca, Spain and strain CA54 was isolated from giant kelp *Macrocystis pyrifera* at Monterey bay, USA. The novel genus *Rosistilla* is formed by three further strains, obtained in our cultivation endeavor: Mal33 (from algae, Mallorca, Spain), CA51 (from giant kelp, Monterey bay, USA) and Poly24 (from our abiotic control). This poster describes the detailed characterization and valid description of both novel genera and the species that constitute them.

## 492-EMP

Fragmented disturbances have an influence on bacterial recolonization processes and activity

\*T. Mainka<sup>1</sup>, A. Worrich<sup>1</sup>, T. Banitz<sup>2</sup>, L. Y. Wick<sup>1</sup>

<sup>1</sup>Helmholtz-Center for Environmental Research, UMB, Leipzig, Germany

<sup>2</sup>Helmholtz-Center for Environmental Research, Leipzig, Germany

**Introduction:** Soil microbial ecosystems are often exposed to environmental disturbances that affect their activity and functional stability.

**Objectives:** Little is known on how the spatial structure of such disturbances affects microbial compound turnover and its functional stability. Previous *in silico* studies of our group (König et al, 2018, Frontiers in Microbiology) suggest that (i) the functional stability and activity of ecosystems exposed to spatially fragmented disturbances is increased and (ii) bacterial motility plays an important role for both speed and extent of recovery.

**Materials & methods:** To challenge these hypotheses, we developed agar-based synthetic microbial ecosystems either allowing for efficient dispersal or hindered dispersal of motile *Pseudomonas putida* KT2440-gfp. Temporally and spatially explicit disturbances of varying spatial configurations were applied by UV-light treatment. Glass fiber networks were used to promote bacterial dispersal and, hence, efficient recolonization of disturbed areas. The number of bacteria and the recolonization of disturbed areas were evaluated by spatially explicit cell counting. The degradation of benzoate was used to measure bacterial functional stability.

**Results:** Our data show an influence of the disturbance fragmentation pattern on the bacterial recolonization process and activity, and hence, confirmed our *in silico* findings: (1) recolonization of habitats exposed to highly fragmented disturbances, recovered faster and exhibited higher functional stability; (2) better bacterial dispersal and functional recovery of the synthetic ecosystem was observed in presence of glass fiber networks.

**Conclusion:** Our results underline the importance of the spatial disturbance patterns and bacterial motility for the functional stability of heterogeneous microbial ecosystems. They further stress the relevance of preferential bacterial dispersal vectors (e.g. given by the presence of fungal mycelia) for sustaining functional stability of disturbed microbial systems.

## 493-EMP

Novel Biodiversity of Nitrate-Reducing Bacteria in Grassland Soils

\*T. Timsy<sup>1</sup>, U. Behrendt<sup>1</sup>, A. Ulrich<sup>1</sup>, T. Spanner<sup>2</sup>, M. A. Horn<sup>2</sup>, S. Kolb<sup>1</sup>

<sup>1</sup>Leibniz centre for agricultural research (ZALF), Microbial Biogeochemistry, Müncheberg, Germany

<sup>2</sup>Leibniz Universität Hannover, Institute of Microbiology, Hannover, Germany

The nitrogen (N) cycle is one of the most complex and environmentally important element cycles on the earth and the key steps are mediated by soil microorganisms. Bacteria can assimilate nitrate for their growth resulting in the formation of organic compounds that are precursors for biosynthesis. Alternatively, nitrate can also be used by bacteria as a respiratory electron acceptor anaerobically and can yield energy by reducing nitrate to either ammonium (dissimilatory nitrate reduction to ammonium, i.e. DNRA) or to N gases by denitrification (i.e. to nitrous oxide or

dinitrogen). DNRA keeps the N available for microorganisms and plants in soil whereas denitrification form N gases that are released to the atmosphere and contribute to N loss in soils. While there is a general agreement about the significance of denitrification in soils, the importance of DNRA is controversially debated, which is mainly attributable to the lack of knowledge on the biodiversity of these bacteria in soils; and thereby, their detectability. Aiming at a substantial increase in organismal biodiversity, we employed massive cultivation coupled with genomics from several grassland soils to reveal new genetic and organismal biodiversity of DNRA bacteria and denitrifiers. We have isolated more than 7,000 bacterial strains and de-replicated using MALDI-TOF MS and 16S rRNA gene sequencing, and we identified more than 700 phylotypes. Based on taxonomic affiliation and experimentally measured N metabolism traits, more than 30 new strains were genome sequenced. As a result, we extended the genetic diversity of genes involved in nitrate/nitrite reduction. Several novel bacterial strains e.g. belonging to the genera *Pseudomonas* and *Shinella*, exhibited physiological traits of nitrate ammonifiers. With the help of the retrieved genome sequences, we aim to improve the understanding of the phylogenetic, metabolic and gene marker diversity of nitrate ammonifiers and denitrifiers.

## 494-EMP

Metabolic activities as possible stability parameter in on demand biogas production

\*W. Getz<sup>1</sup>, F. R. Bengelsdorf<sup>1</sup>, K. Maurus<sup>2</sup>, S. Ahmed<sup>2</sup>, M. Kazda<sup>2</sup>

<sup>1</sup>Institut für Mikrobiologie und Biotechnologie, Universität Ulm, Ulm, Germany

<sup>2</sup>Institut für Systematische Botanik und Ökologie, Universität Ulm, Ulm, Germany

### Introduction & Objectives

A continuous fermentation (CF) of maize silage (MS) and sugar beet silage (SBS) was performed to investigate correlations between metabolic activities and process parameters. The results obtained in this study could contribute to optimize a stable, demand-oriented biogas production.

### Materials & Methods

CF was carried out in four 12 L bioreactors (BR1 to BR4) over 125 days. Amount of MS and SBS were equal in each bioreactor and accounted for 2 kg of volatile solids (VS) per m<sup>3</sup> and d. BR1 served as control and was hourly supplied with MS and SBS. CF was divided into two fermentation phases (1. equilibrium phase and 2. demand-oriented phase). In phase 2, SBS was intermittent SBS supply of SBS was different for all reactors (BR2: at h0, BR3: at h0+h1, BR4: at h0+h1+h2, in period of 12 h). Samples of bioreactor content were drawn weekly to determine enzyme activities of acetate kinase (ACK), isocitrate dehydrogenase (IDH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the crude cell extracts.

### Results

During fermentation phase 2, ACK activity increased in BR2 (from 0.17±0.03 to 0.5±0.17 U mg<sup>-1</sup>) and BR3 (from 0.24±0.07 to 0.68±0.18 U mg<sup>-1</sup>) during the first 9 days of fermentation. The specific methane yield decreased during this period from 333±36 to 241±24 NI kg<sub>VS</sub><sup>-1</sup> in BR2 and from 331±23 NI kg<sub>VS</sub><sup>-1</sup> in BR3. Furthermore, IDH activity in BR1 to 3 increased after 7 days of fermentation (e.g. BR2 0.003±0.001 to 0.016±0.002 U mg<sup>-1</sup>). Furthermore, it was obvious that a pH decrease (from 8.0 to 7.5) may cause an

increased activity for the GAPDH in BR2 (from  $0.02 \pm 0.01$  U  $\text{mg}^{-1}$  to  $0.13 \pm 0.02$  U  $\text{mg}^{-1}$ ) and BR3 ( $0.023 \pm 0.015$  to  $0.135 \pm 0.04$  U  $\text{mg}^{-1}$ ).

## Conclusion

Metabolic activities of microbes react sensitively to a change in the feedstock supply. This was shown by the increased enzyme activities of ACK, IDH and GAPDH during continuous fermentation with altered feedstock supply. The enzymes IDH and GAPDH showed higher activities when pH shifted from alkaline to neutral.

---

## 495-EMP

### Characterization of membrane bound metalloproteins in the anaerobic ammonium-oxidizing bacterium *Candidatus Kuenenia stuttgartiensis*

\*R. Budhraj<sup>1</sup>, S. Karande<sup>1</sup>, C. Ding<sup>1</sup>, S. Wagner<sup>2</sup>, M. Ullrich<sup>2</sup>, T. Reemtsma<sup>2</sup>, L. Adrian<sup>1</sup>

<sup>1</sup>Helmholtz-Centre for Environmental Research GmbH – UFZ, Isotope Biogeochemistry, Leipzig, Germany

<sup>2</sup>Helmholtz-Centre for Environmental Research GmbH, Analytical Chemistry, Leipzig, Germany

Metal ions are abundant in microbial proteins and have many roles in biological system. Metalloproteins are involved in many biological processes and constitute almost one third of total proteins in the living system. In respiration complexes, metalloproteins are often involved in generating a proton motive force across the membrane. Metal ions are well organized in these respiration complexes and form electrically conductive path. A large number of microbial metalloproteins are still uncharacterized. It is important to have a very sensitive, accurate and robust method to study the interaction between metals and proteins. Hence, we adapted and modified a proteomics and metallomics approach for the identification of membrane-associated metalloproteins. A comprehensive metalloproteomics study focused on Fe-, Co-, Ni- and Mo-containing metalloproteins was performed in a culture highly enriched (~90%) with the anaerobic ammonium-oxidizing "Ca. *Kuenenia stuttgartiensis*". The membrane proteins were separated and fractionated under native conditions using one dimensional blue native gel electrophoresis and two dimensional blue native - SDS electrophoresis for the elucidation of subunit compositions in different membrane-bound protein complexes. Proteins and metal ions were identified with liquid chromatography mass spectrometry (LC-MS/MS) and inductively coupled plasma mass spectrometry (ICP-QqQ-MS) coupled with an Aridus desolvator or laser ablation, respectively. We identified several known or yet-undescribed membrane-bound metalloprotein complexes which supports the feasibility of this approach. Our data suggests the presence of Fe, Ni, Zn and Mo binding proteins involved in the anaerobic process of ammonium oxidation such as nitrite reductase, nitrite:nitrate oxidoreductase (NXR), hydrazine synthase (HZS), hydrazine dehydrogenase (HDH), hydroxylamine oxidoreductase (HAO), and NADH ubiquinone oxidoreductase (NUO). These findings reveal a potential coupling of proteomics and metallomics could be used for characterization of in the respiration complexes.

---

## 496-EMP

### Identification of novel glyphosate-protein interactions in *Escherichia coli* revealed by thermal proteome profiling

\*P. Lohmann<sup>1</sup>, A. S. Aldehoff<sup>1</sup>, N. Jehmlich<sup>1</sup>, M. von Bergen<sup>1,2</sup>

<sup>1</sup>Helmholtz-Centre for Environmental Research - UFZ, Molecular Systems Biology, Leipzig, Germany

<sup>2</sup>University of Leipzig, Institute of Biochemistry, Leipzig, Germany

The broad-spectrum herbicide glyphosate (N-(phosphonomethyl) glycine) is the most commonly and intensively used herbicide worldwide. Glyphosate interacts with the enzyme enolpyruvylshikimate-3-phosphate synthase (EPSPS) which belongs to the shikimate pathway and inhibits the *de novo* synthesis of aromatic amino acids phenylalanine, tyrosine and tryptophan in higher plants and microorganisms. In this study, we applied *thermal proteome profiling* (TPP) using the environmental widely distributed bacteria *Escherichia coli* as a model organism. Cultures exposed to glyphosate during growth are subjected to quantitative proteomic analysis to reveal alterations in protein and pathway abundances. Identification of significant melting curve shifts of *E. coli* proteins after incubation with glyphosate should also present new binding partners of the herbicide. We observed that the growth and the viability of *E. coli* decreased with increasing glyphosate concentration. Importantly, we identified four potential new interacting partners of glyphosate because of significant changes in their melting curves and their affiliation to affected pathways. Our study will contribute to a better understanding of the mode of action of glyphosate and encourage the ongoing risk assessment discussions about their application in the environment.

---

## 497-EMP

### Isolation of antibiotic producing species from feces of animals

\*D. Iliasov<sup>1</sup>, T. Mascher<sup>1</sup>

<sup>1</sup>Technische Universität Dresden, Institut für Mikrobiologie, Professur für Allgemeine Mikrobiologie, Dresden, Germany

The rapid spread of antibiotic resistance is considered as a major threat for human and animal health (WHO, 2014). The increasing number of infections with multidrug-resistant bacteria, which are no more treatable with all currently available antibiotics, stresses the need for new antimicrobial compounds even more.

Here, we study cultivable isolates obtained from 35 fecal samples of various animals regarding their potential to produce compounds with antimicrobial and antifungal activity.

Therefore, diluted feces were plated on MYM agar. Seventy potential *Actinobacteria* species were isolated based on the typical colony morphology of *Actinobacteria*. Pure cultures of the isolates were tested regarding their ability to produce antimicrobial substances. For that, well-grown colonies on MYM agar plates were overlaid with soft agar containing *Bacillus subtilis* or *Penicillium chrysogenum* spores. We found antimicrobial activity of thirty isolates against *B. subtilis* or *P. chrysogenum*. In a next step, we tested the antibiotic spectrum of these 30 isolates against several Gram-negative and Gram-positive bacteria as well as against pathogenic fungi, e.g. *Candida tropicalis*.

*B. subtilis* and the fungus *P. chrysogenum* were strongly inhibited by isolates obtained from feces of *Diplodactylus galeatus* (Gecko). The isolates derived from feces of *Pachnoda marginata* *Peregrina* larvae showed an inhibitory effect on the growth of *B. subtilis*, *P. chrysogenum*, *C. tropicalis* and *Exophiala dermatitidis*. One antibiotic-producing isolate that was found in *Grus japonensis*'s feces showed a strong and broad range antibacterial activity against Gram-positive and -negative pathogens.

In future, all isolates, which produced antimicrobial compounds, will be identified and the respective substances

will be further characterized. To conclude, we could demonstrate that feces of animals are a valuable source of bacteria producing promising antimicrobial and antifungal active compounds of biotechnological and medical importance.

---

#### 498-EMP

##### Earthworm-induced bacterial P mobilization by copiotrophic bacteria plays a central role in P turnover in subsoil horizons

\*A. Chiba<sup>1,2</sup>, N. Vitow<sup>3</sup>, C. Baum<sup>3</sup>, A. Zacher<sup>3</sup>, M. Peine<sup>3</sup>, M. Schlöter<sup>1</sup>, P. Leinweber<sup>3</sup>, S. Schulz<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München, Research Unit Comparative Microbiome Analysis, Munich, Germany

<sup>2</sup>Technical University of Munich, Chair of Plant Nutrition, Freising, Germany

<sup>3</sup>University of Rostock, Chair of Soil Science, Rostock, Germany

Phosphorus (P) is one of the essential nutrients for all biota on earth. Microbes are the main drivers for P turnover and control its mobilization from dead biomass as well as from bedrock materials. However, most processes triggering the mobilization of P are highly energy demanding, thus we expect that the corresponding microorganisms will be enriched in hotspots including rhizosphere and drilosphere. Many studies focused on the rhizosphere in the past, while the drilosphere has been rarely studied as a hotspot for P turnover. The drilosphere is defined as the zone in soil, where earthworms induce C accumulation, unlike rhizosphere. We propose that as a result of the C release by the earthworms copiotrophic bacteria increase in abundance and trigger P mineralization processes to maintain stable C:P ratios in the microbial cells. This might be of importance mainly in subsoils where overall concentrations of bioavailable C are low and P might be exclusively mobilized in hotspots. Thus, we collected rhizosphere and bulk soil samples in 10 cm intervals (0-70 cm depth) from a short-term coppice in Rostock, Germany. We combined potential enzyme activities, the quantification, and identification of bacteria involved in P turnover by applying qPCR and amplicon sequencing. We targeted marker genes coding for enzymes involved in mineralization (*phoN*, *phoD* and *phnX*), solubilization (*gcd*), and uptake (*pitA*, *pstS*) of P. High potential enzyme activities for P mineralization were observed in the drilosphere compared to bulk soil in all soil layers, however the differences increased with soil depth as expected. qPCR assays confirmed the higher genetic potential for P turnover in the drilosphere. The abundance of *phoD* gene decreased with soil depth while the genes *gcd* and *pitA* remained high in abundance also in the deeper layers. Each of the marker genes was assigned to different copiotrophic bacteria, such as Bradyrhizobium and Xanthomonas. The heterotrophic bacteria involved in P uptake processes took the most advantage of the earthworm activities in the deeper soil layers, in contrast with oligotrophic bacteria involved in P uptake processes in the bulk soil.

---

#### 499-EMP

##### The fate of 2-heptyl-4-hydroxyquinoline N-oxide in *Pseudomonas aeruginosa* and *Mycobacteroides abscessus*

\*N. Ritzmann<sup>1</sup>, C. Schmerling<sup>1</sup>, S. L. Drees<sup>1</sup>, S. Fetzner<sup>1</sup>

<sup>1</sup>University of Muenster, Institut für Molekulare Mikrobiologie und Biotechnologie, Muenster, Germany

**Introduction** Alkyl quinolones (AQs) are involved in the sophisticated quorum sensing network of *Pseudomonas aeruginosa*. They can contribute to the regulation of

virulence factor production, or serve as antimicrobials. Whereas PQS ("Pseudomonas quinolone signal") is the predominant signaling compound, 2-heptyl-4-hydroxyquinoline N-oxide (HQNO) is a respiratory chain inhibitor. HQNO is detoxified via 3-monooxygenation by various bacteria. The reaction is catalyzed by flavoprotein monooxygenases (FPMOs) and results in PQS-N-oxide (PQS-NO). Interestingly, these FPMOs are highly similar to PqsH, the PQS signal synthase in *P. aeruginosa*.

**Objectives** Since 3-monooxygenation of HQNO is catalyzed by PqsH-like FPMOs in various AQ degraders, HQNO might also be modified by PqsH of *P. aeruginosa*. We hence wanted to investigate the fate of HQNO in *P. aeruginosa* and the AQ degrading *Mycobacteroides abscessus*. Moreover, we aimed to analyze HQNO monooxygenation by PqsH and AqdB, two isofunctional FPMOs of *P. aeruginosa* and *M. abscessus*.

**Methods** We constructed *P. aeruginosa* mutants lacking AQ biosynthetic genes to facilitate detectability of transient HQNO intermediates in biotransformations, and quantified products of HQNO conversion. We purified recombinant proteins and developed a spectroscopic assay to characterize substrate specificities of PqsH and AqdB. We moreover analyzed intermediates of *in vitro* degradation of HQNO by mycobacterial enzymes via LC/MS.

**Results** Supplementation of *P. aeruginosa* with HQNO resulted in formation of a transient PQS-NO intermediate. PQS-NO synthesis was dependent on the presence of PqsH. Remarkably PQS-NO can undergo chemical reduction to PQS in *P. aeruginosa* cultures. Reconstituting HQNO degradation of *M. abscessus* with recombinant proteins *in vitro*, we detected PQS-NO as well as its downstream intermediate N-hydroxy-N-octanoyl anthranilic acid.

**Conclusion** Our experiments indicate that both, the signal synthase PqsH and the AQ degradation enzyme AqdB hydroxylate HQNO besides HHQ. The reaction product PQS-NO therefore may be regarded as an intermediate in both, *P. aeruginosa* QS signal biosynthetic and mycobacterial AQ degradation pathways.

---

#### 500-EMP

##### Enhanced HCH detoxification by anaerobic microbial consortia enriched from HCH-contaminated sediments

M. I. Khan<sup>1,2</sup>, C. Vogt<sup>1</sup>, S. Bashir<sup>2</sup>, \*I. Nijenhuis<sup>1</sup>

<sup>1</sup>Helmholtz-Centre for Environmental Research-UFZ, Department of Isotope Biogeochemistry, Leipzig, Germany

<sup>2</sup>University of Agriculture, Faisalabad, Institute of Soil and Environmental Sciences, Faisalabad, Pakistan

**Introduction:** Organochlorine pesticides such as hexachlorocyclohexanes (HCH) contamination is a serious environmental concern because of their toxicity to biological systems and their persistence in the environment. Among the existing cleanup options, mineralization of HCH by indigenous microorganisms would be the most economical and environmentally-friendly remediation approach for HCH-contaminated sites. Although use of anaerobic microbial consortia has been considered a promising microbial resource for HCH degradation, complete HCH mineralization has yet to be achieved.

**Objectives:** Here, we aim to develop anaerobic HCH-degrading microbial consortia from HCH-contaminated sediments collected from Pakistan, and explore the microbial communities and biodegradation pathways in the newly developed consortia.

**Materials & Methods:** Microcosm experiments were carried out using sediments (collected from two different HCH-contaminated sites in Pakistan) as inocula, sodium lactate as an exogenous C source and e-donor, and one of the HCH isomers (i.e.  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ , each added separately) as e-acceptor.

**Results:** Anaerobic HCH-degrading microbial consortia were successfully enriched through serial sub-culturing. Intermediates analysis by GC-FID showed that the developed consortia had the ability to transform each of the isomers of HCH to monochlorobenzene (MCB) and benzene, particularly with relatively high transformation rates of  $\gamma$ -HCH (i.e. 10-19  $\mu$ M per day). The reduction in MCB and benzene concentrations, at later stages of incubation, indicates the further degradation or mineralization of these transformation products. In addition, the coupling of MCB- and HCH-degrading consortia may further improve the mineralization of HCH.

**Conclusion:** Complete HCH detoxification and mineralization is expected to be feasible under anaerobic conditions.

---

## 501-EMP

### Uncultivated Chloroflexi from fumeroles and hot springs of São Miguel (Azores) harbor unusual large genomes and secondary metabolite clusters

J. Vollmers<sup>1,2</sup>, J. Gescher<sup>3</sup>, \*A. K. Kaster<sup>1</sup>

<sup>1</sup>KIT, IBG 5, Eggenstein-Leopoldshafen, Germany

<sup>2</sup>Karlsruhe Institute of Technology, Institut für Biologische Interfaces (IBG5), Eggenstein-Leopoldshafen, Germany

<sup>3</sup>Karlsruhe Institute of Technology, IAB, Eggenstein-Leopoldshafen, Germany

One of the most "undercultured" phyla is the phylum Chloroflexi, a deep-branching lineage within the domain Bacteria. In its current state, the phylum consists of eight classes (Chloroflexia, Thermomicrobia, Dehalococcoidia, Ktedonobacteria, Ardentocatenia, Thermoflexia, Anaerolineae, and Caldilineae). Interestingly, Chloroflexi isolates exhibit a broad diversity of phenotypes and a wide range of metabolic activities. Recent analyses have shown that they might harbour a great potential for the production of novel secondary metabolites. However, their diverse physiology and metabolism make them often difficult to cultivate. A bioinformatics survey of datasets from publically available databases showed that hot springs all around the world are often hot spots for Chloroflexi species. In this study, samples from fumaroles and hot springs of the Azores were collected, and the extracted DNA was sequenced and bioinformatically analysed. We were able to reconstruct genomes from different metagenomics datasets by covariant binning, resulting in 35 different genomes of previously uncultivated Chloroflexi. The majority seems to represent so far unknown classes within the phylum. In addition, a high number of NRPS/PKS clusters within unusual large genomes of Chloroflexi could be identified, making these bacteria highly interesting for biotechnological applications.

---

## 502-EMP

Accessing the uncultivated marine microbial biodiversity and its novel biomolecules for biofilm prevention and removal

\*M. K. Peters<sup>1</sup>, W. R. Streit<sup>1</sup>, I. Krohn-Molt<sup>1</sup>

<sup>1</sup>University of Hamburg, Department of Microbiology and Biotechnology, Hamburg, Germany

**Background:** Microbial biofilms affect many areas of our daily life, e.g. industry and health care where they cause production failure and infections. As there are no general tools for biofilm prevention or removal available, our project will deliver novel bioactive molecules and technologies to remove biofilms. We specifically aim to isolate proteins and peptides interfering with microbial biofilms. Potent antibiofilm proteins or peptides could offer alternatives to common antibiotics.

**Material and Methods:** Samples of five coral species and water of the shark tank were taken at the Hagenbeck Tropic Aquarium in Hamburg. Enrichment cultures, phylogenomics, metagenome sequencing, binning approaches in combination with functional biofilm assays have been used to identify antibiofilm molecules.

**Results and outlook:** Supernatants from defined enrichment cultures using different corals and shark tank water affected *Stenotrophomonas maltophilia* K279a and *Pseudomonas aeruginosa* PA14 biofilm formation. Preliminary data imply that the mixed cultures derived from the coral *Montipora foliosa* and the shark tank contain biomolecules suitable for biofilm prevention and removal. This was demonstrated using biofilm assays in 96 well microtiter plates. Current work focuses on purification and fractionation of the active fractions in these metagenome cultures.

---

## 503-EMP

### Responses of the soil microbiota to pesticides and their microgel-based adjuvants

\*J. Zierul<sup>1,2</sup>, R. Geer<sup>1,2</sup>, L. Goncharenko<sup>2,3,4</sup>, A. Töpel<sup>2,4,5</sup>, W. Xu<sup>2,4,5</sup>, F. Jakob<sup>2,4,5</sup>, C. Knief<sup>1,2</sup>

<sup>1</sup>University of Bonn, INRES – Molecular Biology of the Rhizosphere, Bonn, Germany

<sup>2</sup>Bioeconomy Science Center, c/o Research Center Jülich, Jülich, Germany

<sup>3</sup>RWTH Aachen University, Institute of Biotechnology, Aachen, Germany

<sup>4</sup>RWTH Aachen University, DWI – Leibniz-Institute for Interactive Materials, Aachen, Germany

<sup>5</sup>RWTH Aachen University, Institute of Technical and Macromolecular Chemistry, Aachen, Germany

The usage of agrochemicals such as fertilizers and pesticides is ubiquitous nowadays. They support the protection of crop plants and ensure a successful growth for a high yield. However, these chemicals can have side effects on the environment, for instance on the soil microbiota. In this study, different agrochemicals, i.e., pesticides and adjuvants were tested for possible environmental influences. Adjuvants are substances which are added to an active ingredient in order to improve its effectiveness. Among the test objects were the fungicide chlorothalonil and the active ingredient copper acetate. In addition, a new adjuvant technology termed *greenRelease* was tested, which aims at a reduction of the amount of agrochemicals that need to be applied. It employs microgels, which act as loadable containers for active ingredients that are fixed onto aerial plant organs via anchor peptides and release the loaded pesticides over time. The different components were applied into soil and samples were taken from the day of the application until 240 days later. Subsequent DNA extraction, 16S rRNA gene amplification and Illumina MiSeq sequencing permitted the detection of potential responses in the bacterial community. Overall, bacterial communities were only little affected by any of the treatments, if at all. Nevertheless, changes in soil bacterial community composition were observed after the application of the ploy(*N*-Vinylcaprolactam) based microgel (pVCL). After a week, bacteria of the genus '*Candidatus Saccharibacteria*' started



to show an increase in relative abundance. These bacteria are a likely candidate to accelerate and ensure the biodegradability of the *greenRelease* adjuvant. Ongoing experiments with fungal communities will complete these analyses soon. Overall, the tested technology *greenRelease* seems promising to enable a reduction of agrochemicals in the future.

---

#### 504-EMP

Surveying extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in urban wetlands in southwestern Nigeria as a step towards generating prevalence maps of antimicrobial resistance

O. O. Adelowo<sup>1,2</sup>, O. O. Ikimiukor<sup>1,2</sup>, C. Knecht<sup>1</sup>, J. Vollmers<sup>3</sup>, M. Bhatia<sup>1</sup>, A. K. Kaster<sup>3</sup>, \*J. A. Müller<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ,

Environmental Biotechnology, Leipzig, Germany

<sup>2</sup>University of Ibadan, Environmental Microbiology and Biotechnology, Ibadan, Nigeria

<sup>3</sup>Karlsruhe Institute of Technology, Institute for Biological Interfaces (IBG5), Karlsruhe, Germany

In many countries, emission of insufficiently treated wastewater into waterbodies appears to be an important factor in the spread of clinically relevant antimicrobial resistant bacteria. Here we queried for the presence of *Enterobacteriaceae* with resistance to 3rd generation cephalosporins in four urban wetlands in southwestern Nigeria via isolation of strains and sequencing their genomes together with qPCR enumeration of marker genes in sediment samples. Genome analysis of multi-drug resistant and potentially pathogenic *Escherichia coli* isolates (members of the broadly distributed ST10 complex) revealed the presence of the extended spectrum beta-lactam resistance gene *bla*<sub>CTX-M-15</sub> on a self-transmissible IncF-type plasmid. The gene was also present together with *bla*<sub>TEM-1B</sub> on a self-transmissible IncH-type plasmid in multi-drug resistant *Enterobacter cloacae* isolates. A *Citrobacter freundii* isolate carried *bla*<sub>TEM-1B</sub> on an IncR-type plasmid without discernable conjugation apparatus. All strains were isolated from a wetland for which previous qPCR enumeration of marker genes, in particular the ratio of *int1* to 16S rRNA gene copy numbers, had indicated a strong anthropogenic impact. Consistent with the isolation origin of the strains, qPCR analysis in this study showed that *bla*<sub>CTX-M</sub> was present at abundance relative to bacterial 16S rRNA gene copy numbers of  $1 \times 10^{-4}$  in sediment samples from that wetland. The results indicate that contamination of the Nigerian urban aquatic ecosystem with clinically relevant antibiotic resistant bacteria could be substantial at some sites. Measures should therefore be put in place to mitigate the propagation of clinically relevant antimicrobial resistance within Nigerian aquatic ecosystems.

---

#### 505-EMP

Does the maize rhizosphere microbial community show spatial variation and dependence on root carbon allocation?

\*S. Schultes<sup>1,2</sup>, R. Koller<sup>2</sup>, R. Metzner<sup>2</sup>, D. Pflugfelder<sup>2</sup>, C. Hinz<sup>2</sup>, M. Watt<sup>1,2</sup>, C. Knief<sup>1</sup>

<sup>1</sup>Bonn University, INRES - Molecular Biology of the Rhizosphere, Bonn, Germany

<sup>2</sup>Jülich Research Center, Institute of Bio- and Geosciences, IBG 2, Enabling technologies, Jülich, Germany

As much as 20% of a crop's photosynthetically fixed carbon is transported belowground where it is used for root growth, respired or released into the rhizosphere. The excretion of organic carbon into the rhizosphere can significantly contribute to plant fitness and soil health. It is a substantial source of soil organic carbon and supports the development

of rhizosphere microorganisms. Although root traits are now increasingly studied, little is known about the temporal and spatial distribution patterns of plant deposited carbon and its effects on the microbial community structure. To address this point, we employed a combination of the two non-invasive imaging techniques Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) to visualize root carbon allocation over time. MRI allows 3D monitoring of root growth in its natural environment, while PET uses the short-lived radioactive tracer <sup>11</sup>C<sub>2</sub> to track carbon fluxes within the roots. Maize plants were grown in a sandy loam for three weeks. Roots were scanned using MRI and PET at day 6, 13 and 20 after sowing. Root growth and <sup>11</sup>C tracer allocation were visualized successfully, revealing an increased tracer accumulation at the root tips. Based on this information, image-guided sampling of the rhizosphere was performed at day 20 after sowing from different root types and areas. Samples of "tracer rich" and "tracer poor" root regions were taken to reveal local differences in microbial community structure. Data of microbial community analysis will be presented, along with the associated tracer allocation patterns obtained by MRI/PET. The non-invasive root imaging techniques are promising tools to monitor root growth and transport processes and define critical time points in root development. The resulting spatiotemporal maps enable targeted sampling of roots plus associated microorganisms to understand root and rhizosphere dynamic processes with spatiotemporal resolution.

---

#### 507-EMP

Importance of redox gradients in hyporheic zones: transformation potential of the recalcitrant pharmaceutical iopromide

\*N. Mukherjee<sup>1</sup>, B. Braun<sup>1</sup>, U. Szewzyk<sup>1</sup>

<sup>1</sup>Technische Universität Berlin, Environmental Microbiology, Berlin, Germany

The hyporheic zone, the interface between surface water and ground water, is known for its importance for the self-purification capacity of rivers. It is characterized by steep redox gradients due to the active mixing of groundwater and surface water. It is very active biogeochemically, with hyporheic passage often resulting in the conversion of nutrients and the attenuation of pollutants. It creates a highly unique environment hosting some highly specialized organisms that provide specific ecosystem functions and services. The microbial community in the hyporheic zone is yet to be properly characterized.

We aim to characterize the correlation between the redox gradient and the microbial community.

Sediments from the hyporheic zone of the Berlin-based river Erpe were transferred to columns in our laboratory. The sediment columns were then fed with a synthetic fresh water medium, a micronutrient solution and iopromide, an iodinated X-ray contrast medium which is a recalcitrant pharmaceutical found in the river Erpe. Pore water samples were then extracted from different depths of the column and analyzed for different parameters such as concentration of dissolved oxygen, different anions, iopromide and its transformation products.

In a first attempt, the column was operated primarily under anoxic conditions. Concentration of iopromide was found to decrease as the feed passes through the column. Over a period of time, pore water samples from the same sampling points reported even lower levels of iopromide. Deiodinated transformation products (TPs) were confirmed in the outlet of the column and no non-deiodinated TPs were seen.



The transformation potential of the sediment from the hyporheic zone could be demonstrated. It is likely that the microbial community in the sediment column adapts to iopromide over a period of time. Further investigation is required to support this claim. The transformation potential of the sediment reactor will be studied further by increasing the amount of oxygen in the initial portion of the gradient.

---

## 508-EMP

Crop rotation and addition of high carbon amendments differentially impact rhizosphere microbial communities

\*C. Kamau<sup>1</sup>, R. van Duijnen<sup>2</sup>, V. Radl<sup>1</sup>, M. Schlöter<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München, Research Institute for Comparative Microbiome analysis, Neuherberg, Germany

<sup>2</sup>University of Lüneburg, Institute of Ecology, Leuphana, Lüneburg, Germany

Due to the growing human population, there is a great need for increase in food production and sustainable management of agro-ecosystems. This is done through implementation of various agricultural management practices which include fertilization using organic amendments and crop rotation. However, the effect of the combined practices on soil microbial communities is not well understood. We set up a 2-year mesocosm experiment to determine the effects of addition of high carbon amendments (wheat straw, sawdust, control) and 2 different crop rotation systems (barley - barley and faba bean - barley) on rhizosphere bacteria communities. We analyzed the bacterial community structure and composition using 16S rRNA gene amplicon sequencing. Our results showed a significantly higher amendment effect (adonis:  $R^2 = 0.565^{***}$ ) on the community composition than the pre-crop effect (adonis:  $R^2 = 0.105^{***}$ ). Ordination analysis of the dataset showed differential clustering of the sawdust amended samples regardless of the pre-crop with the wheat straw and control samples clustering together. Sawdust amended samples showed the highest alpha diversity, species richness and species evenness. In both wheat straw and control amended samples, the relative abundances of bacteria taxa from the *Streptomycetaceae*, *Micromonosporaceae*, *Pseudonocardiaceae*, and *Chitonophagaceae* families were higher. Taxa in these families produce free enzymes which are well suited to degrade the hemicellulose and cellulose components of wheat straw. Moreover, members of *Streptomycetaceae* have been often shown to enhance plant growth and health. On the other hand, there was a significant increase in the relative abundance of taxa from the *Gemmatimonadaceae* and *Xanthobacteraceae* families in the sawdust amended plots. Taxa from these families have been linked to the decomposition of complex soil organic matters. Our study revealed that high carbon amendments had a distinct effect on bacterial community composition in the rhizosphere of crop plants. Addition of wheat straw enriched the soil with bacterial taxa known to confer benefits to plant health.

---

## 509-EMP

DNA extraction bias is more pronounced for microbial eukaryotes than for prokaryotes

\*A. Brauer<sup>1</sup>, M. Bengtsson<sup>1</sup>

<sup>1</sup>University of Greifswald, Institute of Microbiology, Bacterial physiology, Greifswald, Germany

Advances in sequencing technology and paradigm shifts in microbial ecology have led to a prolific rise in studies using SSU rRNA amplicon sequencing to assess microbial communities in various environments. Essential to all of these efforts is preservation and extraction of nucleic acids

from environmental microbial communities. A majority of studies have focused on prokaryotic communities, reflecting the current emphasis on bacteria and archaea in molecular microbial ecology. However, in most natural environments, microbial eukaryotes are abundant, diverse and play essential roles in ecosystem processes. Our aim was to assess whether extraction bias affects microbial eukaryotes at a similar magnitude as prokaryotes in the same environment. We compared the effect of different commercial and custom extraction methods on the perceived community composition of prokaryotes and eukaryotes in marine biofilms growing on seagrass leaves. We used two methods to preserve the DNA in the biofilms prior to extraction with 6 different DNA extraction methods. Microbial community composition was assessed by Illumina MiSeq amplicon sequencing of the SSU rRNA genes of prokaryotes (16S rRNA) and eukaryotes (18S rRNA). Both extraction and preservation method explained a significant amount of variation (PERMANOVA  $p < 0.01$ ) in both eukaryotes and prokaryotes, confirming bias for both groups. However, extraction bias was more pronounced for eukaryotes than for prokaryotes (22% and 17% of variation explained, respectively). Several microbial eukaryotic taxa responded differently depending on extraction method, e.g. Nematodes were underrepresented using mechanical lysis while diatoms were underrepresented using enzymatic lysis. Most microbial DNA extraction methods have been developed and optimized for prokaryotes and may therefore be inadequate for microbial eukaryotes which have a high diversity of cell envelopes posing unique challenges for effective cell lysis and subsequent NA recovery. Based on our results, we recommend careful consideration of sample preservation and DNA extraction method depending on which microbial eukaryotes are present and of interest in the studied environment.

---

## 510-EMP

Functional characterization of the Radical SAM enzyme NirJ involved in heme  $d_1$  biosynthesis

\*H. Meyer<sup>1</sup>, G. Layer<sup>1</sup>

<sup>1</sup>Albert-Ludwigs University of Freiburg, Pharmaceutical Biology, Freiburg im Breisgau, Germany

During denitrification, nitrate is stepwise reduced and released as dinitrogen. Nitrite reductases catalyze the second step of denitrification, namely the one-electron reduction of nitrite to nitrogen monoxide. There are two structurally unrelated dissimilatory nitrite reductases, also distinguishable by their use of different cofactors. NirS, a cytochrome  $cd_1$  nitrite reductase, harbors the tetrapyrrole cofactors heme  $c$  and heme  $d_1$ . Heme  $d_1$  is a dioxoisobacteriochlorin with a central iron ion. Compared to other tetrapyrroles, heme  $d_1$  contains two unusual keto groups at rings A and B, as well as an acrylate side chain at ring D. All biosynthetic steps were elucidated in recent years, except the chemically challenging introduction of both keto groups. The Radical SAM enzyme NirJ might be a possible candidate for this particular reaction. Its gene *nirJ* is located together with other genes required for heme  $d_1$  biosynthesis in the *nir*-operon. NirJ catalyzes the removal of the two propionate side chains at rings A and B [1].

However, it is unknown whether NirJ is also responsible for the subsequent introduction of the two carbonyl functions. Also the mechanism of the propionate removal remains unresolved, because the tetrapyrrole forms lactones at rings A and B under aerobic extraction conditions.

NirJ and its substrate were co-produced and co-purified. Anaerobic extraction of the tetrapyrrole coupled with anaerobic HPLC analysis led to a different retention time of

the tetrapyrroles, compared to aerobically prepared samples, suggesting that the different extraction techniques result in tetrapyrroles with or without lactones. As a next step, anaerobic HPLC-MS analysis could elucidate the true nature of the extracted tetrapyrrole. To establish the complete reaction mechanism, the cleavage product derived from the propionate groups has to be determined. During this cleavage step, a radical intermediate occurs, which might be detectable by EPR spectroscopy. Finally, the stoichiometric concentrations of formed 5"-deoxyadenosine to reaction product should be defined.

[1] L. Boss, R. Oehme, S. Billig, C. Birkemeyer, G. Layer, *The FEBS Journal* **2017**, 284, 4314–4327

## 511-EMP

### Sulfur and Nitrogen Metabolism in Aerobically Growing *Thioalkalivibrio paradoxus*: a Proteome Study

P. Rühl<sup>1</sup>, S. Hein<sup>1</sup>, N. Benker<sup>2</sup>, \*A. Kletzin<sup>1</sup>, C. Lenz<sup>3</sup>

<sup>1</sup>Technische Universität Darmstadt, Mikrobiologie, Darmstadt, Germany

<sup>2</sup>Technische Universität Darmstadt, Institut für Angewandte Geowissenschaften, Darmstadt, Germany

<sup>3</sup>Max-Planck-Institute for Biophysical Chemistry, Bioanalytical Mass Spectrometry Group, Göttingen, Germany

**Introduction:** The halotolerant Gammaproteobacterium *Thioalkalivibrio paradoxus* grows chemolithoautotrophically by aerobic oxidation of thiocyanate (TC), thiosulfate (TS), sulfide, and CS<sub>2</sub> at pH 10 and 35–37°C. Nitrate was added for growth with TS but not with TC.

**Methods:** We conducted a proteomic study of *T. paradoxus* grown with TS versus TC, sampling five time points between 42 and 330 h after inoculation. Quantitative proteomic data were collected from three biological replicates with SWATH-LC/MS/MS.

**Results:** The cultures became transiently milky during exponential phase from extracellular sulfur (S) deposits and intracellular S globules. Later, S disappeared and cell pellets became reddish from cytochromes c.

1951 of 3450 genome-annotated proteins were identified, and the abundances of 1680 (48,7%) determined quantitatively. Proteins of S metabolism were among the five most abundant proteins, including three different B subunits of flavocytochromes c (FccB), and sulfur oxygenase reductase during late exp. and early stat. phases suggesting that the SOR plays a role during oxidation of both extracellular sulfur and intracellular globules. S globule proteins were more abundant with TS than TC. Other abundant proteins included DsrAB (sulfite reductase), while most of the remaining DSR proteins except L were medium-abundant. Surprisingly, the adenylylsulfate reductase was among the most abundant proteins, which together with ATP sulfurylase (Sat) point to a reversely operating sulfate reduction pathway in addition to electron transport chains via DSR proteins, sulfite dehydrogenase (SoeABC), the *bc*<sub>1</sub>-complex, and two terminal oxidases of the *cbb*<sub>3</sub>-type.

TC dehydrogenase was induced with TC as expected, as were CopC, FccB3 and a membrane-bound hydrogenase. Proteins of the incomplete Sox complex were more abundant with TS, as were nitrate and nitrite reductases, and, during stat. phase, CS<sub>2</sub> hydrolase.

**Conclusion:** The results suggest that *T. paradoxus* uses substrate-level and electron transport phosphorylation for ATP formation during aerobic growth with mixed electron

acceptors and aerobic and "anaerobic" electron transport chains.

## 512-EMP

### Spatio-temporal patterns in the root associated microbiota of apple tree

\*M. Becker<sup>1</sup>, C. Knief<sup>1</sup>

<sup>1</sup>University of Bonn, INRES, Bonn, Germany

The rhizosphere harbors highly specific microbial communities, which have the potential to influence plant growth and development. Plants specifically select certain species from the surrounding soil, which thrive in the root-associated soil. Some of these microorganisms even enter the root and establish an endophytic lifestyle. The root system of trees is quite complex and it is assumed that rhizodeposition processes mainly occur at the root tips and in the elongation zone. Older root sections become suberized and thus are considered less relevant concerning carbon release into the rhizosphere and nutrient uptake. It is therefore hypothesized to find different microbial communities along different root regions, which were classified in this study using root diameter as proxy. In order to evaluate these community differences, four commercial apple trees (*Malus domestica* Borkh.) were excavated and the root system was sampled systematically with high spatial resolution and in dependence on root diameter. Furthermore, carbon release into the rhizosphere changes seasonally and thus it is hypothesized that microbial community composition changes seasonally as well. In order to evaluate these seasonal changes of the root-associated microbiota six commercial apple trees were sampled using a core drill over the course of one year. Effects on the root-associated microbiota were evaluated in two compartments: the microorganisms residing inside the roots and closely attached to the root surface were defined as tightly associated, while the microorganisms loosely bound to the rhizoplane and microorganisms found in the rhizosphere were defined as the loosely associated microorganisms. All samples were subjected to bacterial community analysis based on 16S rRNA gene amplicon sequencing. Specific root sections have a stronger impact on endophytic and tightly attached rhizoplane bacterial community composition than on the loosely associated rhizosphere bacteria. The evaluation of the seasonal changes are subject to ongoing experiments.

## 513-EMP

### Synthetic and native consortiums from sewage sludge.

\*G. Angeles De Paz<sup>1</sup>, A. Ledezma-Villanueva<sup>1</sup>, T. Robledo-Mahón<sup>1</sup>, J. Purswani<sup>1</sup>, C. Pozo<sup>1</sup>, C. Calvo<sup>1</sup>, M. Manzanera<sup>1</sup>, E. Aranda<sup>1</sup>

<sup>1</sup>Institute of Water Research, Microbiology, Granada, Spain

More than 700 emerging pollutants are present alone in the European aquatic environment and are considered as potentially serious threats to human health and ecosystems<sup>1, 2</sup>. Environmental law has not regulated them and some are included in the Watch list of potential water pollutants across the EU<sup>2</sup>. Being the pharmaceutical compounds (PhaCs) frequently reported in soils and water bodies causing an important ecological instability<sup>3</sup>. They tend to accumulate in sewage sludge after wastewater treatment and represent the most difficult molecule to degrade due to its high stability<sup>4</sup>. However, some microorganisms have shown the potential to degrade these compounds successfully under specific conditions. Therefore, native fungal and bacterial communities from sewage sludge may play an important role in PhaCs degradation, being able to degrade a myriad of pollutants<sup>5</sup>.

In this work, we performed a microorganism enrichment experiment using sewage sludge from wastewater treatment plants of Granada, Spain, to obtain a native and synthetic inoculum. The composition of the native inoculum was analyzed by Illumina MiSeq, during the selective pressure with carbamazepine, ketoprofen and diclofenac. Then, a synthetic consortium was built using the BSocial online tool (<http://m4m.ugr.es/BSocial.html>). A High diversity of bacteria and fungi was shown inhabits in the sludge. The bioaugmentation of these microorganisms could be regarded as a solution to attend the problem of sewage sludge and other emerging pollutants removal.

## References

1. United Nations Educational, Scientific and Cultural Organization, UNESCO, URL: <https://en.unesco.org/emergingpollutants>
2. The European Commission's science and knowledge service. EU SCIENCE HUB, URL: <https://ec.europa.eu/jrc/en/news/first-watch-list-emerging-water-pollutants>
3. World Health Organization. (2011). (WHO/HSE/WSH/11.05).
4. Gupta R. R., Kumar M., Gupta V. 2013. "Heterocyclic Chemistry: Volume II: Five-Membered Heterocycles". Berlin; Heidelberg: Springer-Verlag.
4. Olicón-Hernández D. R., Camacho-Morales R. L., Pozo C., González-López J., Aranda E. (2019). "Evaluation of diclofenac biodegradation by the ascomycete fungus *Penicillium oxalicum* at flask and bench bioreactor scales". *Science of the Total Environment*. 662: 607-614.

## 514-EMP

Diversity, activity and distribution of microorganisms in the ocean-atmosphere boundary layer

\*I. Hrabec de Angelis<sup>1</sup>, H. M. Aardema<sup>2</sup>, H. A. Slagter<sup>2</sup>, M. L. Calleja<sup>2</sup>, A. Dragoneas<sup>1</sup>, S. E. Ruff<sup>3</sup>, J. Fröhlich-Nowoisky<sup>1</sup>, A. T. Kunert<sup>1</sup>, M. Praß<sup>1</sup>, T. Könnemann<sup>1</sup>, B. Nillius<sup>1</sup>, R. Schiebel<sup>2</sup>, G. H. Haug<sup>2</sup>, E. Thines<sup>4</sup>, U. Pöschl<sup>1</sup>, Z. G. Cardon<sup>3</sup>, C. Pöhlker<sup>1</sup>

<sup>1</sup>Max Planck Institute for Chemistry, Multiphase Chemistry, Mainz, Germany

<sup>2</sup>Max Planck Institute for Chemistry, Climate Geochemistry, Mainz, Germany

<sup>3</sup>Marine Biological Laboratory, Ecosystems Center, Woods Hole, United States

<sup>4</sup>Johannes Gutenberg University Mainz, Institute of Molecular Physiology, Mainz, Germany

Although microbial ecosystems in soil, water and even the deep sea have been extensively studied, the air microbiome has so far received little attention. For climate modelling purposes, the air microbiome above the ocean is usually not considered, although atmospheric microorganisms have been shown to be metabolically active in clouds and to act as efficient cloud condensation and ice nuclei. Hence, the presence and activity of microbes could directly influence atmospheric chemistry, the Earth's radiation budget and hydrological cycles. Furthermore, around 60 % of Earth's surface is covered by clouds, which may be a microbial habitat, not just a mere carrier.

Using the custom-built research sailing yacht Eugen Seibold, we intend to perform contamination-free analysis of a broad range of atmospheric aerosol characteristics in relation to water biogeochemical parameters. Sampling of water- and airborne microorganisms will provide insights into the biotic and abiotic processes occurring in the boundary layer between ocean and atmosphere. An in-house developed fluorescence in situ hybridization protocol (FISH) for atmospheric and oceanic samples will allow the determination of total cell abundance and microbial domain level classification. Microbial community structure will be

assessed using 16S and 18S rRNA gene amplicon sequencing. The twin-plate ice nucleation assay will be used to determine the organisms' capability to act as ice nuclei.

The broad spectrum of instruments and analyses available aboard the Eugen Seibold, including measurements of total atmospheric particle counts and size distribution, atmospheric and water pCO<sub>2</sub> levels, pH, temperature and nutrient levels, together with the microbial community analysis, will allow us to obtain insights into the microbial signature and activity and related processes within the ocean-atmosphere boundary layer. We will investigate the biogeography and dispersal mechanisms of the air microbiome and its potential impact on cloud formation. This work will improve our understanding of the mutual influence of climate relevant parameters and microbial communities.

## 515-EMP

### Halophilic life in gas storage caverns?

\*L. Schwab<sup>1</sup>, P. Bombach<sup>2</sup>, G. Nowack<sup>3</sup>, H. H. Richnow<sup>1</sup>, C. Vogt<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research,

Isotopebiogeochemistry, Leipzig, Germany

<sup>2</sup>Isodetect GmbH, Leipzig, Germany

<sup>3</sup>MicroPro GmbH, Gommern, Germany

The implementation of renewable energy solutions in Germany requires methods for improving their production, storage and distribution. The conversion of power into hydrogen and subsequent storage could balance fluctuations in solar or wind energy. Salt caverns have suitable geochemical properties to serve as a short- and long-term natural gas reservoir. But the effects of energy-rich H<sub>2</sub> storage on microbial structure and function relationships in salt caverns have not been studied yet. We want to determine the biogeochemical processes that might interfere with the storage of H<sub>2</sub> focusing on intrinsic H<sub>2</sub>-oxidizing halophilic microorganisms. Therefore, sump waters of three natural gas reservoirs and one former town gas reservoir, were sampled. Hydro-chemical analyses were done according to ISO norms, investigating the presence of organic and inorganic compounds. Cell numbers were measured based on DAPI staining. Furthermore, pH and temperature were measured during sampling. Sump water analysis revealed detectable amounts sulfate (4.1 ± 0.5 g L<sup>-1</sup>), ammonia (8.6 ± 3.2 mg L<sup>-1</sup>) and nitrate only in 3 of 4 caverns (1.2 ± 0.6 mg L<sup>-1</sup>). Butyrate (44.6 ± 1.6 mg L<sup>-1</sup>) was found in all caverns, whilst acetate (30 mg L<sup>-1</sup>) was only detected in the former town gas cavern. All caverns were characterized by DOC levels above 100 mg L<sup>-1</sup> (292.5 ± 101 mg L<sup>-1</sup>) and corresponding cell numbers ranged from 2.0E+6 to 5.3E+6 cells mL<sup>-1</sup>. Currently, microcosm experiments are running in which sump waters were amended with labelled water (D<sub>2</sub>O) and carbonate (NaH<sub>13</sub>CO<sub>3</sub>) and with H<sub>2</sub> as sole electron donor in order to assess microbial activity and identify active microorganisms by subsequent nanoSIMS analysis. To track biological activity, analyses of H and C stable isotopes in H<sub>2</sub>, CO<sub>2</sub> and CH<sub>4</sub> will be performed using GC-IRMS. For the first time sump waters of different salt caverns were analyzed regarding their suitability as a potential microbial habitat. Cell numbers indicate microbial presence and chemical analysis indicate that short-chain fatty acids and alcohols may play a central role for in-situ microbial activity.

## 516-EMP

Evaluating the effect of chemical compounds on prophage induction in soil and groundwater bacteria

\*K. Parmar<sup>1</sup>, F. Schattenberg<sup>1</sup>, S. Müller<sup>1</sup>, M. Schmidt<sup>2</sup>, L. Y. Wick<sup>1</sup>, R. Kallies<sup>1</sup>, A. Chatzinotas<sup>1,3</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research- UFZ, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Helmholtz Centre for Environmental Research- UFZ, Department of Isotope Biogeochemistry, ProVIS - Centre for Chemical Microscopy, Leipzig, Germany

<sup>3</sup>German Centre for Integrative Biodiversity Research (iDiv) Halle-Leipzig-Jena, Leipzig, Germany

Terrestrial systems are hotspots for unexplored viral diversity and virus-bacteria interactions. Phages (viruses of bacteria) infect bacteria via lytic or lysogenic cycle. The lysogenic cycle is characterized by integration of the phage genome into the bacterial chromosome as a prophage. Prophage get induced by environmental triggers resulting in lysis of hosts and release of new phage in environment. In this work, we studied if chemicals applied in livestock farming or agriculture, and which persist in the environment, induce prophage. Specifically, we analysed prophage induction by the antibiotics ciprofloxacin and trimethoprim, the herbicide glyphosate and mitomycin-C (standard inducer) using model bacteria, 232 isolates from soil and groundwater, and soil communities. Induction was determined by decrease in bacterial optical density (OD) and concomitant increase in phage which were quantified by an optimized flow cytometry (FCM) protocol. To achieve the latter, several parameters were tested with model phages and quantification by FCM was compared to counts by plaque forming units. Further, changes in soil bacterial communities after induction were monitored by T-RFLP. Among the environmental isolates, *Actinobacteria* were predominantly induced among soil isolates, while *Gammaproteobacteria* were mainly induced among groundwater isolates. FCM optimization showed that using TE buffer with 2X SYBR gold and glutaraldehyde fixing of phages resulted in accurate counts. The induction of model organisms by different concentrations of the tested chemicals resulted in distinct patterns: (i) 5 µg/ml ciprofloxacin released maximum number of phages; (ii) phage number increased with increasing trimethoprim concentration; (iii) in contrast, phage numbers decreased as glyphosate concentration increased. Further, T-RFLP revealed changes in community pattern, which were accompanied by higher phage numbers. These results provide evidence that interactions of widely used chemicals with environmental bacteria may result in induction-driven changes of bacterial communities, virus-host interactions and eventually functions.

## 517-EMP

### Anaerobic treatment of manure in biogas plants impacts the release of antimicrobial resistance (AMR) into the environment

S. P. Glaeser<sup>1</sup>, \*E. Aigbekaen<sup>1</sup>, H. M. Khan<sup>1</sup>, D. Pulami<sup>1</sup>, P. Kämpfer<sup>1</sup>

<sup>1</sup>Justus-Liebig-University Gießen, Institut für Angewandte Mikrobiologie, Gießen, Germany

Manure is one of the major sources of antimicrobial resistance (AMR) in the environment, since livestock animals consume the majority of antibiotics produced globally. Antibiotics together with antibiotic resistant bacteria are excreted to the environment via manure, and may significantly contribute to the transmission of and exposure to AMR in food, water, and air. Anaerobic digestion of manure in biogas plants is expected to reduce the release of potential pathogenic and/or antibiotic resistant bacteria into the environment. However to date, simultaneously studied the reduction of all AMR components (antibiotics, bacteria and genes) by this manure intervention are rare.

As part of the international JPI-EC-AMR collaborative project Antimicrobial Resistance Manure Intervention Strategies (ARMIS), we study the effectiveness of manure treatment in

small-scale (farm) biogas plant systems common in Germany with respect to AMR reduction throughout the manure processing. With culture dependent and culture independent methods, the abundance of antibiotic resistance bacteria is determined with the focus on ESBL-producing *Enterobacteriaceae*, vancomycin-resistant *Enterococci* (VRE), Methicillin resistant *Staphylococcus aureus* (MRSA), and Carbapenem-resistant *Acinetobacter baumannii*. First data indicate that the emissions of AMR present in manure is reduced by the anaerobic treatment but not completely stopped. ESBL-producing *E. coli*, VRE, and even strictly aerobic considered bacteria as *Acinetobacter baumannii* survive the anaerobic manure digestion process and are released with the digestate into the environment. Knowledge on the effectiveness of the manure interventions on AMR transmission is an important to contribute efficiently to the reduction of the AMR impact caused by the livestock industry.

## 518-EMP

Apple replant disease: Towards an inoculum with biocontrol properties

\*S. Benning<sup>1</sup>, N. Brugnone<sup>1</sup>, D. Vetterlein<sup>2</sup>, T. Winkelmann<sup>3</sup>, M. Schlöter<sup>1</sup>, V. Radl<sup>1</sup>

<sup>1</sup>Helmholtz Zentrum München, Comparative Microbiome Analysis - Plant Group, Neuherberg, Germany

<sup>2</sup>Helmholtz Zentrum für Umweltforschung, Halle, Germany

<sup>3</sup>Leibnitz Universität, Hannover, Germany

Apple replant disease (ARD) is an issue concerning apple producing areas worldwide. Trees are affected when they are planted in soil, where previously apple or closely related species had been cultivated. The plants show stunted growth, necrotic root tips and lower fruit yield and quality. This is especially a problem for apple orchards and tree nurseries, with many replantings, no space for crop rotation and cost intensively established systems for harvesting and irrigation. Although research is going on for decades, the primary cause for ARD could not be identified by now. Previous experiments pointed towards a lower abundance of many genera of the phylum Actinobacteria, as well as a lower potential for the degradation of phenolic compounds (benzoic acid) in the rhizosphere of replant affected plants. Moreover, it was shown that the effects of ARD on apple seedlings are not systemic and that different microbial communities were found in the soil-root interface of ARD and not ARD plants. Therefore, greenhouse experiments were carried out to enlighten the possible role of Actinobacteria on the apple replant disease. Apple seedlings were grown in rhizoboxes with defined zones of ARD / non-ARD soil. The data revealed a clear difference in the microbiome structure in rhizosphere of replant affected and non-affected soil. In general, Actinobacteria were more abundant in healthy soil compared to ARD soil, except for a few Actinomycetes genera, e.g. *Streptomyces*. Following our findings, we proceeded to isolate Actinomycetes from healthy soil and tested them for their degradation ability of benzoic acid. Of 121 isolates, 21 could degrade the aromatic compound. Their sequences could be assigned to 9 different Actinomycetes genera, *Arthrobacter* sp., *Nocardia* sp., *Pseudarthrobacter* sp., *Terrabacter* sp., and 5 *Rhodococcus* sp. After examining their ability to cope with *Ilyonectria robusta*, a pathogen isolated from ARD affected roots, seven selected strains are being currently tested in a greenhouse experiment for their ability to colonize the roots and to promote the apple tree growth. Thereby, we aim to identify a bacterial inoculum able to mitigate the symptoms of ARD.

## 519-EMP

Cryptic sulfur cycling by cable bacteria in freshwater habitats

\*C. Sachs<sup>1</sup>, D. Kanaparthi<sup>1</sup>, K. As<sup>2</sup>, T. Lüders<sup>1</sup>

<sup>1</sup>University of Bayreuth, Ecological Microbiology, Bayreuth, Germany

<sup>2</sup>University of Bayreuth, Hydrology, Bayreuth, Germany

At diffusion-controlled redox gradients in freshwater sediments, the degradation of organic matter is often limited by the replenishment of electron acceptors. Recently discovered cable bacteria, belonging to the *Desulfobulbaceae* family, can form cm-long filaments, spanning different redox zones and thus able to recycle electron acceptors. They were found to play an important role in oxidative sulphur cycling in marine sediments. For freshwater ecosystems, sulfur cycling is often considered as negligible, due to generally much lower sulfate levels and energetic predictions. However, recent findings suggest that sulfur cycling plays a key role also in certain freshwater habitats, with high turnover rates, but termed "cryptic" due to low steady-state concentrations. The objective of the present study was to connect sulfur cycling in freshwater sediments to long-distance electron transfer (LDET) by cable bacteria, using direct rate measurements involving microsensor profiling to determine sulfide oxidation rates. As sulfide in the suboxic zone cannot be measured while cable bacteria consume it and thus drive the cryptic sulfur cycle, the cycle needs to be interrupted. We incubated sediments derived from lakes and rivers in facilitated diffusion gradient columns to enrich filamentous cable bacteria. Successful enrichment was verified by fluorescence *in situ* hybridization and biogeochemical analyses using microsensors for oxygen, sulfide and pH. For direct rate measurements, the cryptic sulfur cycle was disrupted by preventing LDET by removing the cathodic oxygen supply. Simultaneously, the bacterial community profile was analyzed over depth by next generation sequencing to identify changes in the microbial community composition over depth. Results suggest that cable bacteria constitute a significant but overlooked cryptic sulfur cycling activity in the investigated freshwater sediments.

## 520-EMP

Cues for a microbial link between the cycling of methane and iodine in cave biofilms

\*F. Beulig<sup>1</sup>, C. Karwautz<sup>2</sup>, T. Lüders<sup>1</sup>

<sup>1</sup>University of Bayreuth, Chair of Ecological Microbiology, Bayreuth Center of Ecology and Environmental Research (BayCEER), Bayreuth, Germany

<sup>2</sup>University of Vienna, Department of Limnology & Bio-Oceanography, Vienna, Austria

The cave of an iodine-rich spring in southern Germany is inhabited by extensive microbial biofilms. Microorganisms in these biofilms appear to feed on geogenic exhalations of methane as the primary source of carbon and energy in the cave. Calculated Gibbs free energy yields suggest that the microbial consumption of methane could be driven by the reduction of a variety of different electron acceptors, including O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, Fe(III), and even iodate. Our ongoing investigations focus on (i) exploration of the biofilm metabolic potentials, (ii) discovery of novel methanotrophic taxa, and (iii) identification of postulated metabolic links between the cycling of iodine species and methane. Metagenome sequencing of submersed and subaerial biofilms revealed that microbial communities in the cave were dominated by diverse methanotrophic and methylotrophic taxa, as well as members of the putatively denitrifying, methanotrophic NC10 candidate phylum. De novo assembly and binning of the metagenomic data yielded about 40 near complete genomes harboring diverse metabolic potentials, including complete pathways for methanotrophy, denitrification and haloalkane degradation. Latter may serve as a potential proxy for the microbial turnover of reactive iodine species, including

iodomethane. A planned combination of cultivation and stable carbon isotope tracer experiments will help to identify and cultivate microorganisms potentially involved in the coupled cycling of methane and iodine.

## 521-EMP

Genome comparison of *Candidatus Kuenenia stuttgartiensis* strain WD and strain MBR1 revealed drastic structural changes

\*C. Ding<sup>1</sup>, L. Adrian<sup>1</sup>

<sup>1</sup>UFZ, ISOBIO, Leipzig, Germany

So far there is only one reported complete genome of anammox bacteria – the "*Candidatus Kuenenia stuttgartiensis*" strain MBR1. Here we report another complete genome of the strain WD, which presented as planktonic cells in a semi-continuous stirred-tank reactor (semi-CSTR) with a specific growth rate  $\mu$  of 0.33 d<sup>-1</sup> at 30°C. The genome was obtained through metagenomics sequencing of the reactor culture using a PacBio RSII DNA sequencer. The genome size is 4.33 mbp with a single 16S rRNA gene. The genome of strain WD are very similar with the genome of strain MBR1 in nucleotide sequences and core genes, sharing >99% average nucleotide identity. However the two genomes show enormous differences in genome structures, with four reversed sequences of size between 150 kbp and 500 kbp. By comparing these two genomes we are able to identify essential genes for the anammox organism, and we also tried to explain the cause of the drastic structure change.

## 522-EMP

The author has not agreed to a publication.

## 523-EMP

Water environment as source of non-tuberculous mycobacteria

\*A. Hummel<sup>1</sup>, M. Koch<sup>1</sup>, S. Sandner<sup>1</sup>, \*C. Förster<sup>1</sup>

<sup>1</sup>Umweltbundesamt, Microbiology of Drinking Water and Swimming Pool Water, Bad Elster, Germany

### 1. Introduction

Non-tuberculous mycobacteria (NTM) are ubiquitous organisms, which occur in a wide variety of environmental reservoirs, including municipal and natural water. Surface water has an important role to play for circulation of NTM in the environment, because of NTM detection in different matrices, like biofilms, sediments and water. Due to the fact that one-third of NTM-species are potential pathogens and associated with human disease, these organisms play an increasing role for industrialized countries all over the world.

### 2. Objectives

Application of an improved workflow for isolation and detection of NTM to characterize atypical mycobacteria, which are isolated from different environmental habitats – including various types of surface water, like rivers, dams and wells.

### 3. Materials & methods

Our studies included the evaluation of different techniques for the detection of NTM from untreated water samples and their optimization. This especially applies to the use

of various culture media, incubation temperatures, methods for decontamination and their combination, including further identification steps.

#### 4. Results

Systematic testing of various methods and their development has contributed to improved detection methods for NTM from a wide variety of environmental water reservoirs. We have applied a workflow to maximize the recovery rate with a simultaneous inhibition of the accompanying flora during the isolation of NTM. Further identification of isolated NTM by Mass Spectrometry allowed the characterization of NTM load in natural water habitats.

#### 5. Conclusion

Application of this optimized workflow will be helpful for future analysis of natural water bodies in the course of microbiological risk assessment. NTM can cause disease in humans or animals. Therefore, further characterization of NTM-presence in water environments becomes an increasingly important issue.

---

#### 524-EMP

Improvement of pepper mild mottle virus (PMMoV) analysis in environmental water samples

\*S. Pfeifer<sup>1</sup>, P. Schilbach<sup>1,2</sup>, S. Gläsel<sup>1</sup>, K. Oehm<sup>1</sup>, C. Förster<sup>1</sup>

<sup>1</sup>*Umweltbundesamt, Microbiology of Drinking Water and Swimming Pool Water, Bad Elster, Germany*

<sup>2</sup>*University of Applied Sciences, Mittweida, Germany*

#### Introduction

Monitoring of water sources for the presence of pathogens is fundamental for securing high microbial quality standards for the supply of drinking water.

Several studies have analysed the occurrence of PMMoV in surface water sources as a potential viral indicator for human fecal pollutions. This RNA virus infects a wide range of pepper species worldwide, and thus finds its way into processed food products. Due to its high stability it survives human gut passage intact and was found to be the most abundant virus type in human fecal samples. PMMoV shows several advantages as an indicator for contamination by human feces: it has no pathogenic effect on humans, shows worldwide distribution and is rarely found in animal feces.

#### Objectives

Application of a workflow for isolation, detection and quantification of PMMoV indicated several possibilities for process optimization. We aimed to increase the yield of the reverse transcription of PMMoV RNA, and to improve the robustness of the TaqMan-based qPCR assay.

#### Materials & Methods

The standard methodology for the detection of PMMoV in water samples includes a concentration step, followed by RNA isolation, reverse transcription into cDNA and subsequently quantification by real-time qPCR.

#### Results

The cDNA yield could be increased by an optimized combination of the primers and enzyme for reverse transcription. Further optimizations were performed on the qPCR primer pairs and TaqMan probes. By application of statistical analyses those primer pairs and probes giving highest robustness and lowest limits of detection and quantification within all tested sequences could be identified.

#### Conclusion

By increasing the yield of the reverse transcription of PMMoV RNA, and improving the robustness of the TaqMan-based qPCR assay we could lower the limits of detection and quantification compared to the method described in the literature. Application of this optimized workflow as a new standard will be helpful in future analysis of PMMoV as an indicator for human fecal pollution in water sources, as results will be more accurate and comparability between different studies will be improved.

---

#### 525-EMP

Detection of viable and infectious human adenoviruses in environmental water samples

\*S. Pfeifer<sup>1</sup>, S. Gläsel<sup>1</sup>, K. Oehm<sup>1</sup>, C. Förster<sup>1</sup>

<sup>1</sup>*Umweltbundesamt, Microbiology of Drinking Water and Swimming Pool Water, Bad Elster, Germany*

#### Introduction

Current monitoring of drinking water for the presence of pathogens requires sampling and detection of bacterial indicator organisms to secure the high microbial quality standards. Such classical indicators have drawbacks for the detection of pathogens like viruses, as these are more persistent due to their resistance to environmental stress and can cause infections already at very low concentrations. Thus a risk-based assessment of water quality is required, including the reliable detection of pathogenic viruses in drinking water sources. Human adenoviruses are suited as indicator viruses for such examinations, as they are found in sewage-affected water sources throughout the whole year.

#### Objectives

Quantitative microbiological risk assessment for human adenoviruses requires quantitative methods which can discriminate between viable and non-viable viruses. Standard molecular biology techniques as real-time quantitative PCR will give a quantitative result on the presence of genetic information alone, but not on the viability of detected genomic units. We aimed to establish a robust method for the detection of viable human adenoviruses in water samples and to quantitatively characterize the microbiological quality of several surface water sources utilized for drinking water treatment.

#### Materials & Methods

Our studies included the evaluation of methods for virus enrichment from untreated water samples, followed by virus DNA isolation and real-time qPCR as well as cell-culture techniques for the detection of viable adenoviruses in enriched samples.

#### Results

By using a combination of cell-culture-based methods in combination with molecular biology techniques we were able

to establish a robust workflow for the detection of viable human adenoviruses from untreated water samples. We have applied this workflow on different surface water sources and compared the achieved results with classical methods like the quantitative detection of somatic bacteriophages.

## Conclusion

The obtained results will be used to support future regulatory guidelines on quantitative microbiological risk assessment of surface water sources utilized in drinking water supply.

---

## 526-EMP

### A closer look at the microbiome's dynamics during the start-up phase of biogas fermenters

\*K. Willenbücher<sup>1</sup>, M. Conrady<sup>2</sup>, P. Ramm<sup>2</sup>, A. Schlüter<sup>3</sup>, D. Benndorf<sup>4</sup>, M. Klocke<sup>1</sup>, J. Barrero Canosa<sup>5</sup>, U. Szewzyk<sup>5</sup>

<sup>1</sup>Leibniz-Institut für Agrartechnik und Bioökonomie e.V. (ATB), Department Bioengineering, Potsdam, Germany

<sup>2</sup>Institute of Agricultural and Urban Ecological Projects affiliated to Berlin Humboldt University (IASP), Department of Biogenic Raw Materials, Berlin, Germany

<sup>3</sup>Bielefeld University, Center for Biotechnology (CeBiTec), Institute for Genome Research and Systems Biology, Bielefeld, Germany

<sup>4</sup>Otto von Guericke University, Department Bioprocess Engineering, Magdeburg, Germany

<sup>5</sup>Technische Universität Berlin, Environmental Microbiology, Berlin, Germany

Our study investigates the microbial processes in semi-continuously fed biogas fermenters during anaerobic digestion of lignocellulosic substrates. In our first experiment, we are focusing on the start-up phase. In this initial phase, a functional microbiological community necessary for anaerobic fermentation is formed. Our second experiment will focus on the entire microbial process over the course of one year.

The aim is to characterize the structure and functionality of the microbiome as well as its dynamics during the ongoing biogas processes. By a comprehensive analysis of the community at metagenome, metatranscriptome, metaproteome level and a chemical analysis of the start-up phase, it is possible to identify key organisms as well as key characteristics that provide information about the major factors in the biogas process. A special interest lies in the key species responsible for the hydrolysis of lignocellulosic substrates. In addition, the microbial community will be screened for phages.

An integrated -omics approach including subsequent data set analysis using bioinformatic tools is applied. The analysis of metagenome data sets allows determining both the composition of the microbial communities involved in anaerobic digestion as well as their genetic potential for anaerobic biomass degradation and biomethanization. Pre-characterization is performed by monitoring the structure and development of microbial communities using DNA-based fingerprinting (terminal restriction fragment length polymorphism, TRFLP). The phages are determined by microscopic analyses (transmission electron microscope).

The results of this study will contribute to the understanding of the establishment of functional microbial networks in technical anaerobic digestion processes.

---

## 527-EMP

## Antibiotic-resistant bacteria mussels harvested from the river Rhine

\*N. Zacharias<sup>1</sup>, I. Löckener<sup>2</sup>, S. M. Essert<sup>1</sup>, E. Sib<sup>1</sup>, G. Bierbaum<sup>3</sup>, T. Kistemann<sup>1</sup>, C. Schreiber<sup>1</sup>

<sup>1</sup>Institute for Hygiene and Public Health, University Hospital Bonn, GeoHealth Centre, Bonn, Germany

<sup>2</sup>Institute for Pharmaceutical Microbiology, University of Bonn, Bonn, Germany

<sup>3</sup>Institute of Immunology, Medical Microbiology and Parasitology, University Hospital Bonn, Bonn, Germany

Bacteria and bacterial infections have been treated by antibiotics since the discovery of penicillin in 1928. A worldwide increase in the use of antibiotics led to the emergence of antibiotic resistant strains in almost all bacterial species, which complicates the treatment of infectious diseases. The World Health Organization (WHO) declares the increase in antibiotic resistance as one of the top 10 threats to global health. Several studies show that important pathogenic antibiotic-resistant bacteria can reach the environment by the discharge of sewage treatment plants and combined sewage overflow events. Mussels are used as bio-indicators of heavy metals, chemicals and parasites; they may also be bio-indicators for viruses and bacteria. This study focusses on the influence of treated wastewater on the bacterial flora in the mussels and whether mussels can be used as bio-indicators of the presence of antibiotic-resistant bacteria in water courses.

The river Rhine is a large cross-border-river carrying considerable amounts of wastewater. It rises in the Swiss canton of Grisons and flows through Germany, further through the Netherlands and into the North Sea. In addition to a large diversity of flora it contains a variety of fish and small animal species such as insects, snails and mussels.

The river Rhine is populated by invasive mussel species, such as *Corbicula* spp. and *Dreissena* spp.. Both species are abundant in such high population densities that together, they account for the majority of mussels in the river Rhine.

An influence of the local sewage treatment plant could be shown in regard to the presence of *E. coli* in higher concentrations in the mussel tissue downstream the treatment plant. Antibiotic-resistant bacteria could be found in the tissue of mussels of the genera *Dreissena* and *Corbicula* at different sampling sites of the river Rhine. No multidrug-resistant bacteria could be isolated from the mussel tissue, but were found in samples of the surrounding water body. The present of antibiotic-resistant Gram-negative bacteria in the river Rhine and mussels suggests that some resistance phenotypes are now widespread or even ubiquitous.

---

## 528-MMEP

Modelling of the *Serratia plymuthica* 4Rx13 FPP C-methyltransferase and evaluation of a catalytic mechanism of pre-sodorifen synthesis

\*M. C. Lemfack<sup>1</sup>, W. Brandt<sup>2</sup>, B. Piechulla<sup>1</sup>

<sup>1</sup>Institute for Biological Sciences/University of Rostock, Biochemistry, Rostock, Germany

<sup>2</sup>Leibniz-Institute of Plant Biochemistry, Bioorganic Chemistry, Halle, Germany

Terpenoids are by far the most structurally diverse and dominant class of natural products. They are biosynthesized from C5 isoprene units (isopentenyl pyrophosphate, dimethylallyl pyrophosphate), which are condensed by prenyl pyrophosphate synthases to produce acyclic precursors such as geranyl pyrophosphate (GPP), farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP). These

precursors serve as canonical substrates for terpene synthases to form a broad range of compounds comprising mono- (C10), sesqui-(C15) and diterpenes (C20).

Recently, we showed that several *Serratia plymuthica* isolates produce the unusual polymethylated bicyclic sesquiterpene sodorifen (C16H<sub>26</sub>) [1]. Our studies revealed that a terpene cyclase (*S.p.* SODS) and a C-methyltransferase (*S.p.* FPPMT) are indispensable for the biosynthesis of sodorifen [1,2,3]. The biosynthetic route of this unique sesquiterpene indicates that *S.p.* SODS does not accept FPP as a substrate as generally expected for sesquiterpene synthases. More interestingly, the *S.p.* FPPMT simultaneously catalyzes a methylation at C10 and cyclisation of FPP to produce a five member-ring compound (C16, pre-sodorifen pyrophosphate), which is the substrate of the terpene cyclase in the biosynthesis of sodorifen [3]. It is our interest to unravel the structure of this new *S.p.* FPPMT to better understand the mechanism involved in the synthesis of the C16 precursor.

Based on *ab initio* modelling and threading, using the Robetta Web Server (<http://www.robetta.org>) followed by refinement with YASARA [4] a reasonable 3D protein structure model was created. After docking of SAM and FPP, and subsequent semi-empirical quantum mechanical calculations, an energetically favored mechanism for the formation of pre-sodorifen pyrophosphate was proposed.

To validate this model, site directed mutagenesis studies were performed to determine the role of the amino acids in the active pocket of the enzyme.

[1]. Domik et al., (2016) a FEMS Microbiol Lett.

[2]. Domik et al., (2016) b Front Microbiol.

[3]. von Reuß et al., (2018) JACS.

[4] Krieger et al. (2009) Prot.: Struct., Funct. Bioinf.

Keywords: Modelling, FPP C-methyltransferase, sesquiterpene, pre-sodorifen pyrophosphate, site directed mutagenesis

## 529-MMEP

Elucidation of Dicarboxylic Acid Degradation Including Major Differences to Fatty Acid Degradation in *Cupriavidus necator*

\*C. S. Strittmatter<sup>1</sup>, V. Biesgen<sup>1</sup>, J. Eggers<sup>1</sup>, A. Steinbüchel<sup>1,2</sup>

<sup>1</sup>University of Münster - Institute for Molecular Microbiology and Biotechnology, Münster, Germany

<sup>2</sup>King Abdulaziz University, Environmental Sciences Department, Jeddah, Saudi Arabia

Although the betaproteobacterium *Cupriavidus necator* (formerly *Ralstonia eutropha*) is exhaustively investigated, its  $\beta$ -oxidation is poorly understood. The degradation monocarboxylic fatty acid occurs by  $\beta$ -oxidation. Presumably, dicarboxylic acids degradation is also carried out via the same way. Its elucidation in *C. necator* is complicated by the presence of numerous homologues genes, that all may code for enzymes of  $\beta$ -oxidation pathway.

In previous studies it was shown, that two different gene cluster mediate the degradation of long-chain fatty acids in *C. necator*. Nevertheless, the question occurred, whether dicarboxylic acids are degraded by the same homologues

and whether these obtained clusters manifest themselves in degradation of shorter fatty acids.

While several *Tn5*-mutants showed leaky or no growth with medium-chain dicarboxylic acids adipic and pimelic acid, deletion mutants of the same genes showed no lack of growth. Only the deletion of the Acyl-CoA dehydrogenase B2555 resulted in a leaky growth. To get further insights, proteome analysis via 2D-gel electrophoresis was performed with *C. necator* H16 grown with adipic acid, hexanoic acid or sodium-gluconate as reference. The proteome analysis revealed that adipic acid is degraded via  $\beta$ -oxidation. The cluster B0198-B0200 (3-Oxoacetyl-CoA transferase and  $\beta$ -Ketoadipyl-CoA thiolase) codes for the responsible genes completed with mentioned B2555 and Enoyl-CoA hydratase A3593. Although hexanoic acid degradation also includes A3593, other enzymes responsible differ from the ones executing the dicarboxylic acid degradation. Furthermore, it was shown, that different homologues genes code for the degradation of hexanoic acid, compared to the ones fulfilling degradation of long-chain fatty acids previously shown.

Therefore, we can present the degradation of adipic acid as example for dicarboxylic acids and hexanoic acid as example for medium-chain fatty acids in *C. necator*. Further investigations will show whether the deletion of these genes leads to an abundance of growth or whether other homologues genes will adopt the function.

---

## 530-MMEP

From C1 to C21: A synthetic approach using CO<sub>2</sub> as a carbon source for the production of complex multi carbon products

\*C. Diehl<sup>1</sup>, S. Sundaram<sup>1</sup>, P. Gerlinger<sup>1</sup>, T. J. Erb<sup>1</sup>

<sup>1</sup>Max-Planck-Institute for Terrestrial Microbiology, Biochemistry & Synthetic Metabolism, Marburg, Germany

The rising level of carbon dioxide (CO<sub>2</sub>) in the atmosphere is the subject of investigations for decades in many fields of research. Due to the big impact of changing the climate it is often seen as a threat to human mankind. From a chemical point of view CO<sub>2</sub> is a free available carbon source. Plants and photosynthetic algae convert CO<sub>2</sub> with energy collected from light into glucose. This rather slow process, limited by the slow turnover rate of the Ribulose-1,5-bisphosphat-carboxylase/-oxygenase was already improved by a faster and more efficient synthetic equivalent, the crotonyl-CoA/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle.

Here we show how we develop new pathways for the further conversion of the fixed carbon from the CETCH cycle into complex multi carbon compounds (>C10), such as limonene, bisabolene, farnesene or 6-deoxyerythronolide (6-dEB) by metabolic retrosynthesis. We purify all enzymes needed for the pathways, supply energy equivalents and test them in *in vitro* assays. We demonstrated that metabolic networks with more than 30 enzymes work *in vitro*. Intermediates and products were verified by LC- or GC-MS.

The two fixed carbons from the CETCH cycle are released as the C2-compound glyoxylate. As a first step we convert two molecules of glyoxylate to the C4-compound malate. Therefore we use the  $\beta$ -hydroxyaspartate cycle: Using only one NADPH equivalent per reaction sequence it is the most energy efficient pathway known for glyoxylate assimilation so far. From malate we go either to mevalonate, a precursor for several terpenes and further to limonene, bisabolene or farnesene. To produce 6-dEB, the precursor for the antibiotic drug erythromycin, we feed the fixed carbon back as CoA-esters into the cycle. With this approach we accumulate



propionyl- and methylmalonyl-CoA which are used by the type I polyketide synthase 6-deoxyerythronolide B synthase.

We successfully demonstrated that non-natural complex metabolic networks work *in vitro*. Once efficient synthetic CO<sub>2</sub>-fixing pathways are implemented *in vivo* these pathways offer multiple possibilities to produce complex compounds such as antibiotics and fragrances from carbon harvested from the air.

---

## 531-MMEP

### Genetic standardization and modularization: coming to the rescue of the *in vivo* implementation of synthetic CO<sub>2</sub> fixation pathways.

\*A. Sánchez-Pascuala<sup>1</sup>, T. J. Erb<sup>1</sup>

<sup>1</sup>Max Planck Institute for Terrestrial Microbiology, Biochemistry and Synthetic Metabolism, Marburg, Germany

Nowadays, the depletion of non-renewable resources (*i.e.* fossil fuels) is leading the accumulation of atmospheric carbon dioxide (CO<sub>2</sub>) in a particularly worrying way. The natural biological solution to recapture atmospheric CO<sub>2</sub> is autotrophic CO<sub>2</sub> fixation, which is mainly driven by the Calvin-Benson-Bassham (CBB) cycle, the carbon fixation pathway of photosynthesis. Unfortunately, natural CO<sub>2</sub> fixation has been overwhelmed in its effort to maintain a sustainable carbon cycle to cover human and environmental needs. Recent advances in synthetic biology have provided new ways to harvest atmospheric CO<sub>2</sub> as a sustainable and profitable carbon source. One example is the crotonyl-coenzyme A (CoA)/ethylmalonyl-CoA/hydroxybutyryl-CoA (CETCH) cycle. The CETCH cycle is a synthetic pathway for the fixation of CO<sub>2</sub> that was designed to operate faster and more efficiently than its natural counterpart, the CBB cycle. The CETCH cycle has been fully demonstrated *in vitro*, however, its applicability *in vivo* is still pending and will provide the next step towards the development of synthetic CO<sub>2</sub> fixation processes. Here, we show how to address this *in vivo* challenge by employing genetic standardization and modularization approaches. The whole enzymatic complement of the CETCH cycle will be captured and rewired following the Standard European Vector Architecture (SEVA) and the Modular Cloning (MoClo) system. The genetic/metabolic tool, called standard CETCH (sCETCH), will allow the user-friendly implementation of functional CETCH modules in a wide range of bacteria. The rational design, in combination with a CETCH-auxotrophic *Escherichia coli* reporter strain, will be used to stepwise realize and optimize the CETCH cycle *in vivo*. The successful approach will set the basis for the development and public release of sCETCH as a new standard genetic/metabolic tool for CO<sub>2</sub> fixation.

---

## 532-MMEP

***Clostridium beijerinckii* DSM 6423: Transformation and plasmid-based overexpression of the 1,3-propanediol production gene cluster**

\*T. Schoch<sup>1</sup>, F. R. Bengelsdorf<sup>1</sup>, P. Dürre<sup>1</sup>

<sup>1</sup>Institut für Mikrobiologie und Biotechnologie, Ulm, Germany

## Introduction

1,3-propanediol is a very important platform chemical, which can be used for poly(trimethylene terephthalate) (PTT) production. PTT is a biodegradable polyester, utilized as fibers for carpets or textiles. Microorganisms such as *Clostridium pasteurianum* or *Clostridium beijerinckii* can be used for the fermentation of crude glycerol to 1,3-

propanediol. *Clostridium beijerinckii* DSM 6423 transports glycerol into the cell using a glycerol transporter. In the cell, one mol glycerol can either be oxidized to one mol dihydroxyacetone or reduced to one mol 3-hydroxypropionaldehyde. Dihydroxyacetone gets further metabolized and is used for growth, recovery of reduction equivalents, and production of by-products. For the reductive glycerol degradation pathway, glycerol is reduced to 3-hydroxypropionaldehyde by a glycerol dehydratase. Subsequently, 3-hydroxypropionaldehyde is converted to 1,3-propanediol using a 1,3-propanediol dehydrogenase.

## Material and Methods

In *C. beijerinckii* DSM 6423, genes with the locus tag CLOBI\_51900 and CLOBI\_51910 encode the two subunits of the cobalamin-independent glycerol dehydratase. Downstream of these genes, the 1,3-propanediol dehydrogenase gene (CLOBI\_51920) is located. An overexpression plasmid pMTL85251\_Ppta\_ack\_1,3-propanediol\_CLOBI containing those three genes was constructed using the Ppta\_ack promoter from *Clostridium ljungdahlii* for constitutive gene expression.

## Results and Conclusion

This plasmid was transformed in *C. beijerinckii* DSM 6423 via electro transformation. The effect of the plasmid pMTL85251\_Ppta\_ack\_1,3-propanediol\_CLOBI regarding 1,3-propanediol production by the newly constructed recombinant *C. beijerinckii* [pMTL85251\_Ppta\_ack\_1,3-propanediol\_CLOBI] strain was examined by performing heterotrophic growth experiments using glycerol as substrate.

---

## 533-MMEP

### Designing novel microbiomes to enhance biogas production with metabolic modeling techniques

\*R. Amarante Colpo<sup>1</sup>, B. Henrissat<sup>2</sup>, J. McDonald<sup>3</sup>, F. Centler<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Aix-Marseille University, CNRS, Marseille, France

<sup>3</sup>Bangor University, Natural Sciences, Bangor, United Kingdom

## Introduction

Waste biomass is a source of methane which is formed by the microbial driven process of anaerobic digestion. This process is deliberately facilitated in biogas plants, but also occurs spontaneously in landfill sites. Microbiomes of anaerobic digestion plants are typically originating from animal guts via inoculation with animal manure or slurry. While these microbial communities are already well characterized, microbiomes of landfill sites still lack a more complete understanding, especially as they encounter a more diverse and heterogeneous environment than gut microbiomes.

## Objectives

The SYNBIOGAS project aims at using insights gained during the characterization and analysis of landfill microbiomes, to reach higher digestion rates in industrial digestion plants by using bioaugmentation strategies.

## Methods

We will integrate metagenomic and metatranscriptome data from landfill bioreactors, with enrichment cultures, and isolated microorganisms of landfill sites to characterize novel enzymes and to construct metabolic models as a tool to design synthetic microbiomes with optimal methane production rates. We will create community-wide metabolic networks, reconstruct metabolic network models for individual species, and perform dynamic community simulations. For synthetic community models, the microbial community composition, feedstock composition, and operational parameters will be systematically varied to screen for optimal communities as candidates for experimental bioaugmentation tests. Bioinformatics pipelines will be used including PathwayTools, KBase, COBRA Toolbox, and *µbialSim*.

#### Expected results

Potential modeling results may include (i) suggestion and validation of new species or consortia to be injected into anaerobic digestion plants or landfill sites to improve biogas production; (ii) identification of critical metabolic conversion steps; (iii) identification of species whose activity must be limited to improve biogas production. The communities designed *in silico* will be validated in laboratory tests and tested by industrial partners for their practical application feasibility.

#### 534-MMEP

The author has not agreed to a publication.

#### 535-MMEP

Standalone FMN-dependent nitroreductases from thermophilic bacteria are involved in the formation of coenzyme F<sub>420</sub>

D. Braga<sup>1</sup>, \*M. Hasan<sup>1</sup>, G. Lackner<sup>1</sup>

<sup>1</sup>Friedrich Schiller University, Microbiology, Jena, Germany

**Introduction:** Coenzyme F<sub>420</sub> is a redox cofactor acting as a low-potential hydride carrier in biochemical reactions governing specialized metabolic pathways. It arises interest due to its potential for biotechnological exploitation. In mycobacteria, FbiB catalyzes the two last steps of F<sub>420</sub> biosynthesis. The N-terminal domain of FbiB (CofE) catalyzes the attachment of a polyglutamate tail to the redox core. This domain is shared among all F<sub>420</sub> producers. The C-terminal FMN-binding nitroreductase (NTR) domain was recently shown to reduce the dehydro form of coenzyme F<sub>420</sub> (DF<sub>420</sub>) to its final form. However, the latter domain is absent in other homologs of the enzyme.

**Objectives:** Taking advantage of the fact that genomes of F<sub>420</sub>-producing bacteria also encode standalone NTRs homologous to the C-terminal NTR domain of FbiB, we sought to investigate if they partake the biosynthesis of the mature coenzyme in these microorganisms.

**Methods and results:** An *Escherichia coli* strain able to produce DF<sub>420</sub> was generated and transformed with plasmids encoding the NTRs from *Thermomicrobium roseum*, *Thermorudis peleeae*, and *Sphaerobacter thermophilus*. HRMS analysis revealed the presence of the mature coenzyme F<sub>420</sub> in the intracellular content of each mutant but not in the control strain, where only DF<sub>420</sub> was found. Enzymatic activity of the NTRs was also confirmed *in vitro*, where the hexa-histidine fusion NTRs were incubated with DF<sub>420</sub> and FMN. As a proof-of-principle, we designed an artificial BGC to produce F<sub>420</sub> in *E. coli* consisting of *T. roseum* NTR, and F<sub>420</sub> biosynthetic enzymes from

*Methanocaldococcus jannaschi* and *Paraburkholderia rhizoxinica*. The cultivation of *E. coli* transformed with a plasmid encoding the artificial BGC, indeed, led to the accumulation of coenzyme F<sub>420</sub> decorated with up to four glutamate residues.

**Conclusion:** *In-vivo* and *in-vitro* evidence showed that standalone NTRs can complement the missing C-terminal domain of FbiB in bacteria yielding the mature coenzyme F<sub>420</sub>. Efforts to express customized F<sub>420</sub> BGCs in safe hosts are now possible. The increased availability of coenzyme will facilitate the investigation of F<sub>420</sub>-dependent enzymes in the future.

#### 536-MMEP

##### Biosynthesis of the redox cofactor mycofactocin in *Mycolicibacterium smegmatis*

\*L. A. Peña-Ortiz<sup>1</sup>, A. P. Graça<sup>1</sup>, H. Guo<sup>2</sup>, D. Braga<sup>1</sup>, T. Köllner<sup>3</sup>, L. Regestein<sup>4</sup>, C. Beemelmans<sup>2</sup>, G. Lackner<sup>1</sup>

<sup>1</sup>Friedrich Schiller University, Microbiology, Jena, Germany

<sup>2</sup>Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Junior Research Group Chemical Biology of Microbe-Host Interactions, Jena, Germany

<sup>3</sup>Max Planck Institute for Chemical Ecology, Department of Biochemistry, Jena, Germany

<sup>4</sup>Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Bio Pilot Plant, Jena, Germany

#### Introduction

Mycofactocin (MFT) is a ribosomally synthesized and post-translationally modified peptide (RiPP) that has been proposed by a bioinformatics approach as a novel redox cofactor. It has attracted attention due to the widespread presence of its biosynthetic gene cluster in bacterial and some archaeal genomes, including the causative agent of tuberculosis, *Mycobacterium tuberculosis* (Mtb). Recently it was shown that MFT is involved in ethanol metabolism. While the role of MFT in Mtb pathogenesis is unknown, understanding the structure and biochemical activity of this cryptic cofactor could inspire its utilization as a potential drug target. Although some biosynthetic steps of MFT have been studied *in vitro*, the final structure of MFT remained unknown and the full biosynthetic pathway remained elusive.

#### Objectives

The aim of this study was to identify MFT in the model organism *Mycolicibacterium smegmatis* and to elucidate its final structure. Furthermore, the MFT biosynthetic pathway was to be established *in vivo*.

#### Methods

We used high-resolution LC-MS/MS combined with stable isotope labeling and molecular networking. Genetic studies interrogated the MFT biosynthetic pathway. The cofactor role was shown by activity-based metabolic profiling.

#### Results

We identified a family of congeners of MFT containing up to nine hexose residues, which were determined to be  $\beta$ -1,4-linked glucose units. We detected oxidized and reduced species as well as biosynthetic intermediates of MFT. We also demonstrated that oligoglycosylation is catalyzed by the predicted glycosyltransferase MftF. Furthermore, we showed that the level of MFT increases upon cultivation in ethanol

and we were able to observe cofactor activity using the proposed MFT-dependent carveol dehydrogenase LimC.

## Conclusions

We elucidated the final structure of MFT congeners and established a model of its *in-vivo* biosynthesis in *M. smegmatis*. These results are a milestone for research into the mycofactocin system and will inspire future studies on the occurrence, biochemical function and physiological roles of MFT. Lastly, they can encourage the exploitation of MFT as a drug target or disease marker.

---

### 537-MMEP

#### A T7-based expression system for monitoring transcription and translation *in vivo* in Gram-negative bacteria

\*H. Löwe<sup>1</sup>, A. S. Ortega<sup>1</sup>, K. Pflüger-Grau<sup>1</sup>, A. Kremling<sup>1</sup>

<sup>1</sup>Technical University of Munich, Systems Biotechnology, Garching, Germany

One of the main goals of Synthetic Biology is to turn microorganisms into living machines that can be rationally engineered to perform any desired task, like the synthesis of a metabolite or protein, or the use of a defined set of substrates. To accomplish this, new genes have to be introduced into the microbial host of choice. The exact prediction of the effects of heterologous gene expression remain elusive, however, and often result in reduced growth because of so-called "metabolic burden". In this work we present a new system to measure the effects of over-expression using eGFP as a model protein. By using a mTagBFP2-tagged T7-polymerase for transcription of eGFP DNA, transcription dynamics can be followed *in vivo* by fluorescence, in addition to translation via eGFP fluorescence.

To implement the system, a miniTn7-based integration plasmid for the expression of the T7 polymerase fused to mTagBFP2 was assembled and then integrated into the genome of *Pseudomonas putida* KT2440, our model organism. eGFP under control of the T7-promoter was cloned into a pSEVA-based plasmid and also transferred to *P. putida*. The resulting strain was cultivated in shaking flasks and in microplate reader experiments at different expression levels, and blue and green fluorescence was followed in a spectrofluorometer. By spectral unmixing, fluorescence signals could be separated from the autofluorescence of the cells, revealing the dynamics of transcription and translation *in vivo*. In addition, a simplified, coarse-grained mathematical model of transcription and translation was set up to explain the effects of over-expression showing good agreement with the experimental data.

With this new system and the corresponding model, the dynamics of gene expression can easily be measured on a translational and transcriptional level, unveiling new insights into the involved intracellular processes. On the long run, this method might open up new ways to design and adapt genes for new hosts and accelerate the development of Synthetic Biology.

---

### 538-MMEP

#### Integrating genome-scale metabolic networks in dynamic models of bacterial ecosystems

\*O. Ebenhöf<sup>1</sup>, T. Nies<sup>1</sup>

<sup>1</sup>Heinrich-Heine Universität Düsseldorf, Quantitative and Theoretical Biology, Düsseldorf, Germany

Microbial communities are complex biological systems that are far from understood. Often, the dynamics of microbial ecosystems are described and modelled by generalised Lotka-Volterra (gLV) or Consumer-Resource models (CRM). However, these models describe the growth dynamics of the single microbial species by heuristic equations that depend on the concentration of species and, in the case of CRM, of available nutrient concentrations. We show here how genome-scale metabolic network models can be used to infer key heuristic parameters, such as carbon yield, but also the overall energetics, which then enter dynamic models as parameters. In simple models, we address the question which environmental parameters determine the number of species that may co-exist. Moreover, we quantify how robust stable microbial communities are against invasions of mutants or foreign species. Our combined modelling approach provides a starting point for the development of dynamic models describing ecosystem dynamics based on the underlying metabolism of the single organisms. It is envisaged that these models help to gain insight into material and energy fluxes in microbial ecosystems in general, and thus help clarifying according to which principles metabolic interactions within microbial ecosystems are organised.

---

### 539-MMEP

#### Systematic pathway engineering towards optimized heterologous triterpene production, guided by metabolic modeling in *Synechocystis* sp. PCC 6803

\*A. Germann<sup>1</sup>, M. Dietsch<sup>1</sup>, D. Moser<sup>1</sup>, S. Triesch<sup>1</sup>, I. M. Axmann<sup>1</sup>

<sup>1</sup>Heinrich-Heine-University, Synthetic Microbiology, Düsseldorf, Germany

Triterpenes and triterpenoids build a large class of almost ubiquitous natural products with a broad structural diversity, hence gaining increased importance in pharmacology as well as in plant sciences and molecular biology, to name but a few.

Since the synthesis of those structures is a chemically highly sophisticated task, it is far from any industrial application. Therefore, the use of genetically modified microorganisms provides a sustainable alternative for large scale plant triterpenoid synthesis. Especially cyanobacteria, as autotrophs, are a promising resource for the synthesis of renewable, sustainable fuels and other high carbon compounds. One effort to overcome initially low yields of heterologous expression is constraint-based flux analysis, a mathematical approach to identify target reactions for modification to enhance product formation, using linear optimization.

The current study is focused on the cyanobacterial MEP-pathway, and the production of cyclized oxidosqualene metabolites. To evidently review the modification targets suggested by modeling, key reactions in the MEP-pathway were enhanced by overexpression. Afterwards, the triterpene-directed metabolite flux was compared with the model predictions. Preliminary results, confirmed by GC-MS, show drastically enhanced triterpene yield when SQS, the precursor of all triterpenoids, is synthesized above native rates, indicating a possible bottleneck. The tested strains with the highest increase in triterpenoid synthesis also displayed impaired growth as well as reduced pigment concentrations. Furthermore, a unique blue phenotype was observed.

In summary, the presented results validate the computational predictions, and the created strains could introduce promising platforms for increased synthesis of triterpenoids.

---

#### 540-MTP

Anoxic cell rupture of *Prevotella bryantii* by high-pressure homogenization protects the Na<sup>+</sup>-translocating NADH:quinone oxidoreductase from oxidative damage

\*L. Schleicher<sup>1</sup>, G. Fritz<sup>1,2</sup>, J. Seifert<sup>3</sup>, J. Steuber<sup>1</sup>

<sup>1</sup>University of Hohenheim, Microbiology, Stuttgart, Germany

<sup>2</sup>University of Freiburg, Neuropathology, Freiburg im Breisgau, Germany

<sup>3</sup>University of Hohenheim, Animal Science, Stuttgart, Germany

Respiratory NADH oxidation in the rumen bacterium *Prevotella bryantii* is catalyzed by the Na<sup>+</sup>-translocating NADH:quinone oxidoreductase (NQR). We describe a protocol to disrupt *P. bryantii* cells under exclusion of O<sub>2</sub> using the EmuLiFlex-C3 homogenizer (Avestin), which leads to fourfold higher NQR activity compared to the oxic preparation.

---

#### 541-MTP

The author has not agreed to a publication.

---

#### 543-MTP

K<sub>d</sub>-value determination of DctP<sub>Am</sub> – a substrate binding protein of the tripartite ATP-independent periplasmic transport system in *Advenella mimigardefordensis* DPN7<sup>T</sup>

\*L. Schäfer<sup>1</sup>, C. Meinert-Berning<sup>1</sup>, A. Steinbüchel<sup>1,2</sup>

<sup>1</sup>Institut für Molekulare Mikrobiologie und Biotechnologie, Westfälische Wilhelms-Universität Münster, Biology, Münster, Germany

<sup>2</sup>King Abdulaziz University, Environmental Sciences Department, Jeddah, Saudi Arabia

In this study, a tripartite ATP-intependent periplasmic transport (TRAP) system in the biotechnologically interesting Gram-negative betaproteobacterium *Advenella mimigardefordensis* DPN7<sup>T</sup> was investigated. In general, TRAP systems are composed of a binding-protein-dependent three-component (DctM, DctQ and DctP) transport system, associated with two regulatory components designated as DctS and DctR. The interactions between the periplasmic binding-protein DctP and ligands like gluconate were investigated.

Therefore, site-directed mutagenesis was performed to get specific amino acid exchanges in the binding pocket of DctP. 13 amino acid exchange mutants were generated. Additionally, DctP<sub>Am</sub> (MIM\_c39430) and the generated variants were purified after heterologous expression of *dctP* in *Escherichia coli* BL21 using the pET19b vector. Thermal shift assays were performed using different ligand concentrations (2.5 µM to 2000 µM) and 1 µM DctP<sub>Am</sub>. In addition to the thermal shift assays, K<sub>d</sub>-values were determined using fluorescence spectroscopy method. The fluorescence was measured at 328 nm with a PMT voltage of 350-380 V. After volume correction and normalization the K<sub>d</sub>-values were determined using values for the fluorescence decrease [%] plotted to the substrate concentration [µM].

In comparison to other SBPs of TRAP transport system, DctP<sub>Am</sub> accepts its ligands with similar affinities. Usually, the SBP proteins show very low K<sub>d</sub>-values for one or two substrates and only show strongly decreased affinity to some other ligand. K<sub>d</sub>-values of DctP<sub>Am</sub> and the generated variants were determined to analyze the impact the different amino

acid exchanges on the substrate binding. Gluconate, galactonate, erytronate, fuconate, and xylonate as ligand for the SBP. The K<sub>d</sub>-values calculated for the wild type protein DctP<sub>Am</sub> were in the range of 2-8 µM with the different ligands. In most cases, the K<sub>d</sub> of the generated variants are higher than the K<sub>d</sub> of the wild type DctP<sub>Am</sub>. In some cases, no K<sub>d</sub>-value could be determined. Especially the variants G39V, R154A, R175K and F198I led to an increase of the K<sub>d</sub> with most ligands.

---

#### 544-MTP

Structure-function relationships in the transporter and sensor kinase CbrA of *Pseudomonas putida* KT2440

\*L. Wirtz<sup>1</sup>, M. Eder<sup>1</sup>, H. Jung<sup>1</sup>

<sup>1</sup>LMU, Microbiology, Martinsried-Planegg, Germany

The transporter and histidine kinase CbrA together with the response regulator CbrB of *Pseudomonas putida* KT2440 represent a two-component system that regulates the intracellular carbon/nitrogen balance and is involved in carbon catabolite repression. CbrA contains a complete transporter domain with similarity to sodium/substrate symporters (SLC5 family) fused to cytoplasmic histidine kinase domains via a STAC domain. We set out to further explore the functional properties and interactions of the individual domains of CbrA. For this purpose, we deleted individual domains of CbrA and analyzed the impact of the deletion on the expression of known target genes in *P. putida* KT2440 using reporter gene fusions. In addition, we genetically engineered, expressed, and purified individual domains and truncated versions of CbrA and compared the functional properties with wild type CbrA. Functional analyses involved transport measurements and the detection of kinase and phosphor transfer activities with radiolabeled substrates. We found that the SLC5 domain of CbrA of *P. putida* KT2440 catalyzes the uptake of histidine into the cell. We analyzed substrate specificity, kinetics and energetics of the transport process. We demonstrated that CbrA transport activity depends on proton motive force and is specific for histidine. Furthermore, we showed that CbrA is able to phosphorylate itself and to transfer the phosphoryl group to CbrB. His766 of CbrA was experimentally proven to represent the site of phosphorylation in CbrA. So far we could not detect an impact of the SLC5 domain on the activities of histidine kinase domains. Similarly, there was no significant effect of the latter domains on transport catalyzed by SLC5. In conclusion, transport and signal transduction seem to be independent processes in CbrA.

#### References

Nishijyo, T., et al. (2001) *Mol. Microbiol.* **40**, 917-931.

Sepulveda, E. & Lupas, A.N. (2017) *Front. Microbiol.* **8**, 2287.

---

#### 545-MTP

Structural and functional analysis of bacterial phospholipid biosynthesis enzymes from *Xanthomonas campestris*

\*G. Vasilopoulos<sup>1</sup>, R. Moser<sup>1</sup>, C. Simon<sup>1</sup>, M. Aktas<sup>1</sup>, F. Narberhaus<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Microbial Biology, Bochum, Germany

Recent studies revealed that membrane composition and lipid biosynthetic pathways in plant-interacting bacteria are different from *Escherichia coli*. Bacterial membranes usually

consist of phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL) [1]. Some bacteria, such as *Xanthomonas campestris*, also synthesize phosphatidylcholine (PC), an important lipid for host-microbe interactions [2]. Previous studies targeted two 1-acyl-sn-glycerol-3-phosphate acyltransferases (AGPAT) Xc\_0188 and Xc\_0238 in *X. campestris*, being responsible for PC synthesis via a novel acylation pathway [3]. Purification of AGPATs in their active form is challenging. The difficulty derives from their tendency to lose activity after solubilization from their native membrane environment into detergent micelles. In this study, the role of Xc\_0188 and Xc\_0238 in lipid metabolism was further characterized. Therefore, protocols for the purification of both enzymes in their active form were established. In agreement with the previous results, Xc\_0188 and Xc\_0238 lead to PC synthesis upon overproduction in *E. coli*. Contrary to our expectations, *in vitro* analysis with purified Xc\_0238 revealed a PlsC like activity, acylating lyso-phosphatidic acid to phosphatidic acid (PA), rather than a PC synthase. PA is a precursor for the synthesis of various membrane glycerophospholipids. Our findings followed by further research can give more biochemical and structural insights into the enzymatic characteristics of AT.

[1]. Sohlenkamp, C & Geiger, O (2016) Bacterial membrane lipids: diversity in structures and pathways. *FEMS Microbiol Rev.*

[2]. Aktas, M et al. (2010) Phosphatidylcholine biosynthesis and its significance in bacteria interacting with eukaryotic cells. *Eur J Cell Biol.*

[3]. Moser, R et al. (2014) Phosphatidylcholine biosynthesis in *Xanthomonas campestris* via a yeast-like acylation pathway. *Mol Microbiol.*

#### 546-MTP

Biochemical characterization of phospholipid *N*-methyltransferases from the thermophilic actinobacterium *Thermobifida fusca*

\*J. Kleetz<sup>1</sup>, A. S. Mizza<sup>1</sup>, M. Aktas<sup>1</sup>, F. Narberhaus<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Microbial Biology, Bochum, Germany

The phospholipid phosphatidylcholine (PC) is a common component of eukaryotic membranes and has thus been studied extensively. The role and biosynthesis of PC in bacteria, however, is only poorly understood until now [1]. Interestingly, many PC-synthesizing bacteria interact with eukaryotic hosts, like the plant pathogen *Agrobacterium tumefaciens* [2]. One of the main pathways for bacterial PC-biosynthesis is the methylation pathway. Here, phospholipid *N*-methyltransferases (Pmts) catalyze the *S*-adenosyl methionine (SAM)-dependent methylation of the phosphatidylethanolamine (PE) headgroup to monomethyl-PE, dimethyl-PE and finally to PC. The three methylation steps can either be catalyzed by a single Pmt or by the consecutive action of multiple enzymes. Until now, the only bacterial Pmt that has been characterized *in vitro* is PmtA from *A. tumefaciens*, catalyzing all three methylations from PE to PC [3,4].

In the present study, we aim to unveil the biochemical properties of newly discovered Pmts. Bioinformatic analyses suggested two putative Pmts (Tfu\_0154 and Tfu\_0735) in the thermophilic actinobacterium *Thermobifida fusca*. Tfu\_0154 displayed Pmt activity *in vivo* as well as *in vitro* upon purification, almost exclusively methylating PE to monomethyl-PE. Tfu\_0735 carries an amino acid substitution in the conserved SAM-binding site, presumably preventing

activity. This study contributes to a more detailed understanding of bacterial Pmts, highlighting the differences to previously characterized enzymes.

#### References

[1] Sohlenkamp, C; Geiger, O (2016). *FEMS Microbiol Rev* **40**, 133–159.

[2] Wessel, M; Klüsener, S et al. (2006). *Mol Microbiol* **62**, 906–915.

[3] Aktas, M; Narberhaus, F (2009). *J Bacteriol* **191**, 2033–2041.

[4] Danne, L; Aktas, M et al. (2015). *Mol Microbiol* **95**, 313–331.

#### 547-MTP

A Na<sup>+</sup> A<sub>1</sub>A<sub>0</sub> ATP synthase with a V-type c subunit in a mesophilic bacterium

\*D. Litty<sup>1</sup>, V. Müller<sup>1</sup>

<sup>1</sup>Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt, Germany

**Introduction:** ATPases/ATP synthases are present in all domains of life and arose from a common ancestor. The *in vivo* function of bacterial F<sub>1</sub>F<sub>0</sub> and archaeal A<sub>1</sub>A<sub>0</sub> ATP synthases is ATP synthesis, whereas eukaryal V<sub>1</sub>V<sub>0</sub> ATPases are incapable of ATP synthesis and are designed by nature to energize intracellular membranes by ATP hydrolysis. The evolutionary switch from synthase to hydrolase is thought to be caused by a doubling of the rotor subunit c, followed by a loss of the ion binding site.

**Objective:** To challenge the hypothesis that a V-type c subunit causes loss of ATP synthesis.

**Material & methods:** The ATPase of *Eubacterium limosum* was purified to apparent homogeneity by classical chromatography and reconstituted into proteoliposomes in order to determine ATP synthesis.

**Results:** Inspection of the genome sequence of the bacterium *E. limosum* revealed the presence of genes encoding an archaeal ATP synthase and, most interestingly, the predicted c subunit gene is of the V-type. The ATPase was purified to apparent homogeneity containing all 9 subunits. Notably, the apparent molecular mass of the c subunit (14 kDa) matched the predicted mass (15.7 kDa) indicating that the c subunit arose by gene duplication and subsequent fusion of the genes. The c subunit contains one conserved Na<sup>+</sup> binding motif and biochemical analysis verified the Na<sup>+</sup> dependence of ATP hydrolysis. The c subunit-directed inhibitor DCCD inhibited ATP hydrolysis, but inhibition was relieved by addition of Na<sup>+</sup>. Na<sup>+</sup> was shown directly to abolish binding of the fluorescence DCCD derivative, NCD-4, to subunit c, demonstrating a competition of Na<sup>+</sup> and DCCD/NCD-4 for a common binding site. After incorporation of the A<sub>1</sub>A<sub>0</sub> ATP synthase into liposomes, ATP-dependent primary transport of <sup>22</sup>Na<sup>+</sup> as well as ATP synthesis driven by an electrochemical Na<sup>+</sup> potential could be demonstrated.

**Conclusion:** The Na<sup>+</sup> A<sub>1</sub>A<sub>0</sub> ATP synthase from *E. limosum* is the first ATP synthase with a 4 TMH c subunit for which ATP synthesis could be proven with a purified enzyme. This

disproves the hypothesis that a V-type c subunit is the cause for the inability of V-type ATPases to synthesize ATP.

#### 548-MTP

##### Fragment-based drug discovery: a crystallographic approach for antibiotics development for gram-negative bacteria

\*D. Stegmann<sup>1</sup>, J. Vering<sup>1</sup>, M. Marsh<sup>2</sup>, J. Steuber<sup>1</sup>, G. Fritz<sup>1</sup>

<sup>1</sup>University of Hohenheim, Cellular Microbiology, Stuttgart, Germany

<sup>2</sup>Swiss Light Source, Villigen, Switzerland

The sodium pumping NADH: Quinone Oxidoreductase (Na<sup>+</sup>-NQR) is an important respiratory enzyme in many gram-negative bacteria (1), often found within pathogenic bacteria like *Acinetobacter baumannii*, *Pseudomonas aeruginosa* or *Vibrio cholerae*. Inhibiting the Na<sup>+</sup>-NQR (2) is a new approach for antibiotic research, which we are pursuing using fragment-based drug discovery (FBDD). FBDD is a technique used to identify small molecular weight lead molecules in a structure-based drug development pipeline. The three-dimensional structures of protein targets (3) in combination with the derived chemical properties in regions of interest are used to develop tailor made compounds binding with high affinity and specificity to the target protein. The recent progress in data collection and crystal handling makes X-ray crystallography-based lead identification a competitive and fast method for high throughput screening of libraries of possible binding molecules. The pipeline of this approach starts by the identification of low molecular weight compounds of approximately 200 Da – so called fragments-via high throughput screening of an optimized crystal system. These crystals are incubated with potential fragments dissolved in DMSO. Subsequent X-ray analysis allows to determine binding and the location of the fragments. The lipophilic nature and small size of the fragments makes them likely to bind to hydrophobic areas of the protein that often represent substrate binding pockets or reaction centers. Fragments identified can be modified and combined to yield follow-up compounds with higher affinity and specificity to the target. Typically, drug development includes several rounds of optimization and modification. With the crystallization of the FAD-domain of the F-subunit of NQR of *V. cholera* (4) we have been able to establish a crystal system suitable for this task at the Swiss Light Source synchrotron and have so far screened over 100 fragments. This has already resulted in several initial hits for follow-up work.

(1) doi: 10.1515/hsz-2014-0204

(2) doi: 10.1016/j.micpath.2013.12.002

(3) doi: 10.1098/rsta.2018.0422

(4) doi: 10.1038/nature14003

#### 549-MTP

*Acinetobacter baumannii* phospholipases D are involved in biosynthesis of cardiolipin and monolysocardiolipin

\*K. Pfefferle<sup>1</sup>, B. Averhoff<sup>1</sup>

<sup>1</sup>Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt, Germany

**Introduction:** The multidrug resistant human pathogen *Acinetobacter baumannii* is a leading cause of hospital acquired infections worldwide. Lipid rich cell membranes are upon the first barriers encountered by the pathogen. Phospholipases play an essential role in the lipid metabolism

and have already been identified as virulence factors [1]. *A. baumannii* produces three phospholipases D (PLD) which act in a concerted manner during infection and support invasion of lung epithelial cells [2].

**Objectives:** The first goal was to elucidate the role of the three PLDs in modulation of lipid composition in *A. baumannii*. The second goal was to get insights into the role of the conserved catalytic motifs in the function of PLD3.

**Materials & methods:** Total lipids of single, double and triple *pld*-deletion mutants were analyzed via TLC and MALDI-TOF. The functional essential histidines in the catalytic motifs were exchanged against aspartate or alanine and total lipids of the variants were analyzed via TLC.

**Results:** A *pld1* mutant did not produce monolysocardiolipin (MLCL) whereas a *pld2,3* and a *pld1,2,3* mutant lacked both, cardiolipin (CL) and MLCL. Members of the PLD family share the presence of two copies of a conserved catalytic HxKx4Dx6GSxN motif. The PLD3 of *A. baumannii* contains one conserved and one modified HKD-motif. In the latter the conserved aspartate is replaced by tyrosine. Mutant studies revealed that the histidine residues of the conserved and the modified HKD-motif are essential for the function of PLD3 in CL and MLCL biosynthesis.

**Conclusion:** PLD1 plays a role in MLCL biosynthesis, whereas PLD2 and PLD3 are important for both, CL and MLCL formation. The absence of CL and MLCL in the total lipids of a *pld1,2,3* mutant producing PLD3 variants leads to the conclusion that both HKD-motifs in the PLD3 are important for CL and MLCL production.

1. Schmiel, D and Miller, V. 1999. *Microbes Infect* **1**: 1103-1112

2. Stahl, J, Bergmann, H, Göttig, S, Ebersberger, I, Averhoff, B. 2015. *PloS One* **10**: e0138360

#### 550-MTP

Comparing methods to study the uptake route of radiolabeled ADEP derivatives

\*A. Vorbach<sup>1</sup>, H. Brötz-Oesterhelt<sup>1</sup>

<sup>1</sup>University of Tuebingen, Interfaculty Institute for Microbiology and Infection Medicine - Microbial Bioactive Compounds, Tuebingen, Germany

The increasing amount of multidrug-resistant pathogenic bacteria has caused an urgent need for novel antibiotics. In the process of characterizing and improving novel antibacterial agents it is crucial to clarify the uptake mode and the pathway of uptake into the target pathogen.

One well described way of studying antibiotic uptake is the tracking of radiolabeled derivatives. After exposure of the bacteria to the labeled antibacterial agent of interest and removal of surplus label by washing, the radioactivity associated with the treated bacteria can be measured by a photomultiplier with the help of scintillation liquid.

The standard way of separating treated bacteria from the surrounding medium is filtering them through membranes. A major problem of this workflow is that for some promising compounds there are no membranes with fitting properties available. Despite extensive washing, free compound may still adhere to the membrane causing high radioactive background values.

Here, we highlight the usefulness of silicon oil to separate the radioactive compound trapped inside the bacteria from the surplus compound in the surrounding medium. In our study, we were able to easily separate radiolabeled ADEP derivatives trapped in bacteria from surplus ADEP in the surrounding medium by using silicon oil, whereas an extended collection of filter membranes had resulted in high background values.

---

### 551-MTP

The antibiotic negamycin permeates across the cell envelope of *E. coli* by multiple routes

\*D. Hörömpöli<sup>1</sup>, C. Ciglia<sup>2</sup>, K. H. Glösenkamp<sup>3</sup>, H. Falkenstein-Paul<sup>4</sup>, G. Bendas<sup>4</sup>, A. Berscheid<sup>1</sup>, H. Brötz-Oesterhelt<sup>1</sup>

<sup>1</sup>University of Tübingen, IMIT, Microbial Bioactive Compounds, Tübingen, Germany

<sup>2</sup>University of Duesseldorf, Institute for Pharmaceutical Biology, Duesseeldorf, Germany

<sup>3</sup>Squarix GmbH, Marl, Germany

<sup>4</sup>University of Bonn, Institute for Pharmaceutical Chemistry II, Bonn, Germany

The increase of multidrug-resistant bacterial pathogens is one of the main health issues of today. Especially treatment of Gram-negative bacteria is challenging, as permeation of molecules across the multiple barriers of the Gram-negative envelope is limited. To better understand the routes that nature has invented for entry into the bacterial cytoplasm, we study the translocation pathways of a natural product antibiotic.

Negamycin is a pseudo-dipeptide antibiotic with promising activity against Gram-negative and Gram-positive bacteria, including Enterobacteriaceae, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and good efficacy in infection models (1,2). It binds to ribosomes with a novel binding mode, stimulating miscoding and inhibiting ribosome translocation (3). No cross-resistance with other ribosome-binding antibiotics was observed. We investigated the passage of negamycin across the cell envelope using *Escherichia coli* as a model.

Our results show that negamycin is able to cross the cell envelope by more than one route. We could identify multiple transporters that are involved in the translocation of negamycin across the cytoplasmic membrane. Furthermore, uptake is influenced by the energy state of the membrane.

The example of negamycin demonstrates that passage across the bacterial cell envelope can be multi-faceted, even for a single small agent and that for cytoplasmic anti-Gram-negative drugs understanding of entry process and target interaction are equally important.

(1) Hamada *et al.* J. Antibiotics 23, 170-171 (1970)

(2) McKinney *et al.* ACS Infect. Diseases 1, 222-230 (2015)

(3) Polikanov *et al.*, Mol. Cell. 56, 541-550 (2014)

---

### 552-MTP

**The membrane anchor of TatB primarily interacts with TatB, not with TatC**

\*M. H. Werner<sup>1</sup>, H. Geise<sup>1</sup>, T. Brüser<sup>1</sup>

<sup>1</sup>Institut für Mikrobiologie, Leibniz Universität Hannover, Hannover, Germany

The twin-arginine translocation (Tat) system of prokaryotes and plant plastids serves to transport folded proteins across energized biological membranes. In *Escherichia coli*, the most intensively studied model organism for Tat transport, three components, namely TatA, TatB, and TatC, assemble to functional Tat translocons. It is well known that TatB stably interacts with TatC, thereby forming the core of the Tat translocon that also interacts with TatA. Early studies suggested a 1:1 interaction for TatB with TatC in purified Tat translocons [1]. The interaction site for the TatB membrane anchor with TatC has been identified at the fifth trans-membrane helix of TatC. However, additional TatB interaction sites are likely, and transcriptional analyses supported an excess of TatB over TatC, implying additional interactions of TatB that may even not be interactions with TatC. We therefore placed the artificial UV-inducible cross-linker amino acid *p*-benzoyl phenylalanine (Bpa) at several positions of the TatB membrane anchor and analyzed its interactions *in vivo*. Notably, the results indicated that TatB almost exclusively cross-linked with itself. This was confirmed by non-specific cross-linking with formaldehyde and glutaraldehyde. In any case, the cross-links indicated homo-oligomers and therefore self-associations *in vivo*. The data therefore strongly question the believe that TatB exclusively interacts with TatC *in vivo*. The ability of TatB to homooligomerize has been demonstrated [2], and it now rather appears that TatB primarily exists in such homooligomeric associations that may well be associated with TatC at specific TatB-TatC interaction sites.

[1] Bolhuis *et al.* (2001) TatB and TatC Form a Functional and Structural Unit of the Twin-arginine Translocase from *Escherichia coli*. *J. Biol. Chem.* **276**, 20213–20219

[2] Behrendt J, Brüser T. (2014) The TatBC complex of the Tat protein translocase in *Escherichia coli* and its transition to the substrate-bound TatABC complex. *Biochemistry* **53**, 2344-2354

---

### 553-MTP

The author has not agreed to a publication.

---

### 554-MTP

**The Mechanism behind Bacterial Lipoprotein Release: Phenol-Soluble Modulins Mediate Toll-Like Receptor 2 Activation via Extracellular Vesicle Release from *Staphylococcus aureus***

\*K. Schlatterer<sup>1</sup>, \*C. Beck<sup>1</sup>, D. Kretschmer<sup>1</sup>

<sup>1</sup>Eberhard Karls Universität Tübingen, Infektionsbiologie, Tübingen, Germany

The innate immune system uses Toll-like receptor (TLR) 2 to detect conserved bacterial lipoproteins of invading pathogens. The lipid anchor attaches lipoproteins to the cytoplasmic membrane and prevents their release from the bacterial cell envelope. How bacteria release lipoproteins and how these molecules reach TLR2 remain unknown. *Staphylococcus aureus* has been described to liberate membrane vesicles. The composition, mode of release, and relevance for microbe-host interaction of such membrane vesicles have remained ambiguous. We recently reported that *S. aureus* can release lipoproteins only when surfactant-

like small peptides, the phenol-soluble modulins (PSMs), are expressed. Here we demonstrate that PSM peptides promote the release of membrane vesicles from the cytoplasmic membrane of *S. aureus* via an increase in membrane fluidity, and we provide evidence that the bacterial turgor is the driving force for vesicle budding under hypotonic osmotic conditions. Intriguingly, the majority of lipoproteins are released by *S. aureus* as components of membrane vesicles, and this process depends on surfactant-like molecules such as PSMs. Vesicle disruption at high detergent concentrations promotes the capacity of lipoproteins to activate TLR2. These results reveal that vesicle release by bacterium-derived surfactants is required for TLR2-mediated inflammation.

### 555-SeSiP

A role for metabolic flux sensing in second messenger control

\*M. van Berkum<sup>1</sup>, A. Reinders<sup>1</sup>, S. Ozaki<sup>1</sup>, M. Sangermani<sup>1</sup>, B. Sellner<sup>1</sup>, U. Jenal<sup>1</sup>

<sup>1</sup>University of Basel, Biozentrum, Basel, Switzerland

The second messenger cyclic-di-GMP (c-di-GMP) is a ubiquitous regulator of bacterial growth and behavior. A total of 25 enzymes synthesizing or degrading c-di-GMP are encoded in the *E. coli* K-12 genome. These enzymes are thought to have different input signals for their expression or activity, resulting in spatiotemporal c-di-GMP control. Yet little is known about potential heterogeneity of this second messenger within populations. Recently we found evidence that *E. coli* populations can display bimodal expression of the c-di-GMP phosphodiesterase pdeL. PdeL expression is negatively regulated by c-di-GMP levels and critically depends on the global transcriptional regulator Cra. Because Cra activity correlates with glycolytic flux, the regulator was proposed to act as metabolic flux sensor (Kochanowski et al. 2013).

To investigate the role of Cra and of metabolic flux distributions in bimodal pdeL expression, we generated a ligand-binding-deficient Cra mutant, Cra\*. Loss of ligand binding was demonstrated by electrophoretic mobility shift (EMSA) and thermal shift assays (TSA). Strains harboring the cra\* allele showed reduced expression of pykF, a gene that is negatively controlled by Cra, as compared to wild type. Interestingly, this difference was abolished at low glycolytic flux conditions. The mutated regulator significantly altered protein levels of multiple Cra targets. We also show that pdeL expression can be elevated under glycolytic conditions in a cra\* background.

The Cra-dependent expression of pdeL shows a direct link between central carbon metabolism and second messenger control. Because bimodal pdeL expression could drive bimodal c-di-GMP concentration distributions within *E. coli* populations, we hypothesize that this could be part of a bet-hedging strategy to direct differential behavior in constantly changing environments. We are currently investigating what the role of metabolic flux is, via Cra and PdeL, in c-di-GMP heterogeneity within bacterial populations.

### 556-SeSiP

Cyclic nucleotides in Archaea: Cyclic di-AMP in the archaeon *Haloferax volcanii* and its putative role

\*F. Braun<sup>1</sup>, L. Thomalla<sup>1</sup>, C. van der Does<sup>1</sup>, T. E. F. Quax<sup>1</sup>, T. Allers<sup>2</sup>, V. Kaefer<sup>3</sup>, S. V. Albers<sup>1</sup>

<sup>1</sup>Universität Freiburg, Biologie II, Freiburg i. Br., Germany

<sup>2</sup>University of Nottingham, School of Life Sciences, Nottingham, United Kingdom

<sup>3</sup>Hannover Medical School, Research Core Unit Metabolomics, Hannover, Germany

Second messengers are crucial components for the perception of environmental stimuli for all living organisms. One major class of these second messengers are cyclic nucleotides. The role of these molecules within intracellular signal transduction has been well studied in bacteria. One quite recently discovered bacterial second messenger is c-di-AMP. It has been demonstrated to be essential in bacteria, pointing towards its vital function for the cell. Compared to bacteria, significantly less is known about cyclic nucleotide second messengers in archaea. This study represents the first direct evidence of the *in vivo* presence of c-di-AMP in an archaeon.

The euryarchaeal model organism *Haloferax volcanii* was demonstrated to produce c-di-AMP under various growth conditions by performing LC-MS/MS on cell pellet extracts. The only diadenylate cyclase encoded in the genome of *H. volcanii* belongs to the DacZ class. Using thin-layer chromatography and [ $\alpha$ -<sup>32</sup>P]-labeled ATP as substrate it could be shown that the *H. volcanii* DacZ enzyme produces c-di-AMP *in vitro*. Similar to bacteria, the *dacZ* gene is essential and homologous overexpression of DacZ leads to cell death, suggesting the need for tight regulation of c-di-AMP levels, which indicates that it is involved in the control of important regulatory processes. A major target of c-di-AMP signaling in bacteria is the maintenance of the cellular osmotic homeostasis. The results presented here suggest a comparable function in *H. volcanii*. An exchange of the *dacZ* gene's promotor resulted in a mutant strain with significantly decreased c-di-AMP levels compared to the wild type. This strain exhibited a characteristic growth phenotype of increased OD-values, especially in media with low salt concentration. Light-microscopical analysis unveiled that the increased OD-values were not caused by elevated growth but rather by a considerably increased cell volume. This implies an impaired osmoregulation in this strain.

In summary, this study expands the field of c-di-AMP research to archaea. Additionally, it indicates that the regulation of osmohomeostasis is likely to be a common function of c-di-AMP signaling in bacteria and archaea.

### 557-SeSiP

Control of cyanobacterial day-night metabolism by the output regulator of a biological clock in *Synechocystis* sp. PCC 6803

\*N. Scheurer<sup>1</sup>, C. Köbler<sup>1</sup>, A. Wilde<sup>1</sup>

<sup>1</sup>University of Freiburg, Institute of Biology III, Freiburg, Germany

Cyanobacteria exhibit rhythmic gene expression with a period length of about 24 hours to adapt to daily environmental changes. In the model organism *Synechococcus elongatus* PCC 7942 (hereafter *Synechococcus* 7942), the central oscillator consists of the three proteins KaiA, KaiB and KaiC and utilizes the histidine kinase SasA and its response regulator RpaA for output-signaling. In *Synechococcus* 7942 the circadian clock system is rather well understood, whereas circadian networks of other cyanobacteria can be very diverse and are less exhaustively investigated. It is still controversial, whether the model strain *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis* 6803) exhibits a true circadian clock. While *Synechococcus* 7942 is obligate photoautotrophic,



*Synechocystis* 6803 can also grow chemoheterotrophically in the dark. However, *Synechocystis* 6803 harbors homologs of the Kai proteins and the output pathway. The *rpaA* deletion mutant showed reduced viability in light-dark rhythms and chemoheterotrophic growth in the dark was abolished completely. Transcriptomic data revealed that RpaA is mainly involved in the regulation of genes related to C<sub>i</sub>-acclimation in the light and to carbon metabolism in the dark. Specifically, genes involved in the metabolism of the storage compound polyhydroxybutyrate (PHB) are upregulated in the dark in the wild type but not in the  $\Delta rpaA$  strain. Furthermore, we performed preliminary measurements of the carbon storage compounds PHB and glycogen. In  $\Delta rpaA$  cells that were grown under high carbon conditions, the glycogen metabolism and synthesis of PHB seemed to be affected compared to the wild type. Thus, we suggest a connection of RpaA to the carbon metabolism of *Synechocystis* 6803. In summary, we show a wide-ranging involvement of RpaA in the regulation of the metabolism of *Synechocystis* 6803 in a diurnal light-dark rhythm.

### 558-SeSiP

Mechanism and function of the non-standard circadian clock system KaiB3C3 in *Synechocystis* sp. PCC 6803

\*C. Köbler<sup>1</sup>, A. Wiegard<sup>2</sup>, I. Axmann<sup>2</sup>, A. Wilde<sup>1</sup>

<sup>1</sup>University of Freiburg, Institute of Biology III, Freiburg, Germany

<sup>2</sup>Heinrich Heine University Düsseldorf, Institute for Synthetic Microbiology, Düsseldorf, Germany

Through the rotation of Earth, all organisms are subjected to daily environmental changes. To facilitate their adaption, many organisms generate an internal rhythm with a period length of around 24 hours, referred to as circadian rhythm. Within cyanobacteria *Synechococcus elongatus* sp. PCC 7942 functions as a model organism for the circadian clock. Its clock consists of three core proteins: KaiA, KaiB and KaiC. Phosphorylation and dephosphorylation of KaiC maintains the timing mechanism. *Synechocystis* sp. PCC 6803 contains two additional homologs of the *kaiB* and *kaiC* genes. To our knowledge, no function for the non-standard clock KaiB2C2 has been described so far; however, Aoki and Onai [1] suggested that KaiC3 and KaiB3 modulate the amplitude and period of the main oscillator KaiAB1C1. In line with this, our *in vitro* and yeast two-hybrid data strongly imply cross talk between the non-standard clock KaiB3C3 and the main oscillator KaiAB1C1. Subsequent analysis of KaiC3 showed a reduced ATPase activity of the clock protein not only in the presence of KaiB3, but also in the presence of KaiB1. In contrast to the inactivation of the main oscillator ( $\Delta kaiAB1C1$ ), deletion of *kaiC3* had no effect in light-dark cycles, yet showed growth impairment during chemoheterotrophic growth in complete darkness [2]. Thus, emphasizing the function of KaiC3 and KaiB3 in acclimation to darkness. While the main oscillator KaiAB1C1 occupies the histidine kinase SasA and its response regulator RpaA for signal transduction, KaiB3C3 utilizes an RpaA-independent output pathway. A putative candidate for the KaiB3C3 output-signaling pathway is the NarL-type response regulator SII0485. However, direction of signaling and additional players will be investigated in future experiments.

1. Aoki, S. & Onai, K. *Bacterial circadian programs*. Ch. 15, 259-282 (Springer Berlin Heidelberg, 2009).
2. Wiegard, A., Köbler, C., Oyama, K., Dörrich, A. K., Azai, C., Terauchi, K., Wilde, A., and Axmann, I. *Synechocystis KaiC3 displays temperature and KaiB dependent ATPase activity and is important for viability in darkness*. 2019. bioRxiv. doi: <https://doi.org/10.1101/700500>

### 559-SeSiP

**From insects to plants: about the fact of insect pathogenic *Photorhabdus luminescens* in the rhizosphere.**

\*A. Regaiolo<sup>1</sup>, N. Dominelli<sup>1</sup>, S. Eckstein<sup>1,2</sup>, R. Heermann<sup>1</sup>

<sup>1</sup>JGU Mainz, Microbiology, Mainz, Germany

<sup>2</sup>Ludwig-Maximilians-Universität München, Biozentrum, Microbiology, München, Germany

*Photorhabdus luminescens* are Gram-negative enteric bacteria that live in close symbiosis with soil nematodes, are in turn highly pathogenic against insects and exist in two phenotypic cell forms. Phenotypic heterogeneity in bacterial cell populations allows genetically identical organisms to different behavior under similar environmental conditions. The two phenotypic different cell variants of *P. luminescens* are referred to as symbiosis phenotypic variant (primary or 1° cells) and symbiosis "deficient" phenotypic variant (secondary or 2° cells). 1° and 2° cells are genetically identical and equally pathogenic towards insect larvae. However, they differ in diverse phenotypic traits, 1° cells display: i) toxins, extracellular enzymes and pigments production; ii) secondary metabolites like antibiotics and bioluminescence; iii) clumping factor iv) occurrence in crystalline inclusion proteins; the majority of which are missing or have a reduced level in 2° cells. Moreover, the big divergence is represented by the success of the *P. luminescens* relationship with entomopathogenic nematodes (ENPs): 2° cells can neither grow in ENPs nor support their development. It is assumed that 2° cells could adapt to a free lifestyle in soil, hence better respond to different stress environmental conditions, nutrient poverty and plant derived molecules like those in the rhizosphere. Therefore, phenotypic switching in *P. luminescens* has to be tightly regulated since it could lead the bacterium to live in different environments: alone in the rhizosphere or in association with ENPs. Here, we present the main regulatory mechanisms important for phenotypic switching in *P. luminescens* cell populations and discuss the biological reason as well as the fate of the 2° cells in the rhizosphere, especially their effect towards plant roots. Understanding the role and the function of the bacteria in the rhizosphere accelerates the progress in biocontrol manipulation and agricultural management strategies.

### 560-SeSiP

**Reporter gene systems for the investigation of the TPP riboswitch as a novel target for antibiotic substances**

\*A. Hübenthal<sup>1</sup>, M. Mack<sup>1</sup>, M. Mayer<sup>2</sup>

<sup>1</sup>Hochschule Mannheim, Institute for Technical Microbiology, Mannheim, Germany

<sup>2</sup>Ruprecht-Karls-Universität Heidelberg, ZMBH, Heidelberg, Germany

Riboswitches are noncoding RNA elements located in the 5'-untranslated region of mRNAs. They function as genetic switches and modulate downstream gene expression upon binding of a specific ligand. The most widespread bacterial riboswitch appears to be the one responding to thiamine pyrophosphate (TPP), which is an essential cofactor for carbohydrate metabolism. Upon binding of TPP, the riboswitch affects the expression of thiamine synthesis and transport genes in a negative feedback loop. Nucleotide sequences and functions of TPP riboswitches are conserved amongst bacteria, including key ESKAPE pathogens. The common occurrence of riboswitches in bacteria and the fact that they are not found in humans qualifies them as a potential drug target in a range of bacterial pathogens.

The goal of the project is to set up an *in vivo* testing system for the TPP riboswitch to identify new potential antibiotic

substances. To reach this goal, translational and transcriptional reporter gene fusions of TPP riboswitches from various bacteria shall be created. Expression of these reporter gene fusions in *Escherichia coli* will validate that binding of potential riboswitch ligands indeed interferes with gene expression. The corresponding ligands will be identified in a high-throughput-screening. As a reporter gene, the *E. coli*  $\beta$ -galactosidase gene *lacZ* will be used, which allows detection as well as quantification of gene expression. By using translational and transcriptional fusions, the operating principle of the respective riboswitch can be characterised as well. Subsequently, the affinities of the identified ligands to TPP riboswitches of different bacteria will be analysed. It will be investigated, whether the novel potent compounds have comparable effects on riboswitches of various bacteria, especially strains known to cause nosocomial infections. Furthermore, the mode of action of the new ligands, as well as possible enzymatic modifications, shall be analysed.

With these approaches, the antibiotic potential of newly identified thiamine analogs can be examined. This will provide an important basis for the development of urgently needed new antibiotics with novel targets.

### 561-SeSiP

The role of stable and dynamic piston regions for transmembrane signaling by sensor kinase DcuS of *E. coli*

\*M. Stopp<sup>1</sup>, P. A. Steinmetz<sup>1</sup>, G. Unden<sup>1</sup>

<sup>1</sup>Johannes Gutenberg-Universität, Institute of Molecular Physiology, Mainz, Germany

The C<sub>4</sub>-dicarboxylate (C<sub>4</sub>DC) metabolism in *E. coli* is regulated by the DcuS/DcuR two component system [1]. The membrane-integral sensor histidine kinase DcuS is activated by periplasmic C<sub>4</sub>DC (such as fumarate) resulting in cytoplasmic autophosphorylation [2]. Due to physical separation of signal input and output (phosphorylation) by the membrane a signal transfer mechanism is required. Signal transduction across the membrane includes a piston type shift of transmembrane helix 2 (TM2) [3], but the complete mechanism for transmembrane and cytoplasmic signal transmission is unknown.

Homodimerization of TM2 and of adjacent regions in the inactive and the fumarate activated state was investigated by scanning the reactivity of DcuS single cysteine variants, revealing the dimerization interface. The kinetics of cysteine reactivity of selected interface residues were performed in presence and absence of the effector fumarate. The data disclose static and dynamic parts of the DcuS dimer during signal transduction. Conspicuous regions were tested for significance for DcuS function by alanine/cysteine substitutions in a *dcuB-lacZ* reporter gene assay which revealed a SxxxGxxxG interaction motif in TM2 and its significance for signal transduction in DcuS.

### References:

[1] Zientz et al. 1998. J. Bacteriol. 180: 5421-5

[2] Scheu et al. 2010. Future Microbiol. 5: 1383-402

[3] Monzel and Unden 2015. Proc. Natl. Acad. Sci. 112: 11042-7

### 562-SeSiP

### Regulation of the fructose lysine metabolism in *Escherichia coli*

\*F. Koller<sup>1</sup>, D. Gast<sup>2</sup>, A. Hoffmann-Röder<sup>2</sup>, J. Lassak<sup>1</sup>

<sup>1</sup>Ludwig-Maximilians-Universität, Department of Biology, Martinsried, Germany

<sup>2</sup>Ludwigs-Maximilians-Universität, Department of Chemistry, München, Germany

Nε-Fructosyllysine (FrK) is a Maillard sugar produced by the non-enzymatic reaction of glucose and lysine. As a posttranslational protein modification it plays a role in diabetes mellitus as well as aging processes in the human body. *Escherichia coli* can grow on FrK as a sole carbon source. The corresponding metabolic proteins are encoded in an operon *frlABCD*. Upon uptake of FrK by the permease FrlA, the amino sugar is decomposed into lysine and glucose-6-phosphate by the concerted enzyme activities of the kinase FrlD and the deglycase FrlB. In contrast to the decomposition, the genetic regulation is not yet understood. Bioinformatic analyses and studies on the *Bacillus subtilis* FrK degradation pathway suggest that the putative transcription factor FrlR plays a pivotal role here. Employing *PfrlABCD-Lux* reporter fusions of varying length we succeeded in unmasking FrlR as a road block repressor and identifying the corresponding operator sequence. Using microscale thermophoresis and surface plasmon resonance spectroscopy, we confirmed our finding *in vitro* and also determined DNA binding affinity. A structural model identifies FrlR as a GntR-like repressor consisting of an UTR substrate binding domain and a Helix-Turn-Helix-DNA binding domain. In summary, our data lead to a regulatory model in which dimeric FrlR blocks the transcription of the *frlABCD* operon. Interestingly, FrK is not recognized directly but only after phosphorylation by FrlD. Binding of Frk leads to structural rearrangements in the FrlR transcriptionally active dimer and liberates the operator. As additional levels of regulation we found catabolite repression by the cAMP-responsive transcription activator CRP and heat stress-induced expression by the alternative sigma factor  $\sigma$ 32. This complexity enables optimal adaptation to intestinal growth where FrK is present as a component of human food.

### 563-SeSiP

The phosphoglucosamine mutase GlmM moonlights in modulating the activity of the diadenylate cyclase CdaA in *Listeria monocytogenes*

\*R. Bremenkamp<sup>1</sup>, J. Heidemann<sup>2</sup>, J. Gibhardt<sup>3</sup>, R. Seifert<sup>4</sup>, V. Kaever<sup>4</sup>, R. Ficner<sup>2</sup>, F. M. Commichau<sup>3</sup>

<sup>1</sup>Institut für Mikrobiologie und Genetik, Allgemeine Mikrobiologie, Göttingen, Germany

<sup>2</sup>Institut für Mikrobiologie und Genetik, Molekulare Strukturbiologie, Göttingen, Germany

<sup>3</sup>Institut für Biotechnologie, Synthetische Mikrobiologie, Senftenberg, Germany

<sup>4</sup>Institut für Pharmakologie, Research Core Unit Metabolomics, Hannover, Germany

The second messenger cyclic diadenosine monophosphate (c-di-AMP) is essential for Firmicutes under most growth conditions. It is produced by diadenylate cyclases (DACs) and degraded by phosphodiesterases. The opportunistic human pathogen *Listeria monocytogenes* possesses only the DAC CdaA. The understanding of the regulation of the activity of CdaA could be a stepping point for the development of novel antibiotics. The gene encoding CdaA is localized in a highly conserved *cdaA-cdaR-glmM* operon. The *cdaR* gene encoding the membrane-bound CdaR protein is known to regulate CdaA. The *glmM* gene encodes the phosphoglucosamine mutase GlmM, which is essential for cell growth because it produces cell wall precursors. The genetic conservation of the *glmM* gene suggests that GlmM might also be involved in the regulation of the DAC activity of

CdaA. The aim of this study was to elucidate the effects GlmM has *in vivo* on CdaA in *L. monocytogenes*. For this purpose, mass spectrometry analysis to determine the intracellular c-di-AMP concentration as well as growth tests under varying osmotic stress conditions were performed. The localization of the proteins was analyzed by employing Western blot analysis. It could be shown that GlmM has an inhibitory effect on CdaA upon hyperosmotic shock, which leads to a strong decrease of intracellular c-di-AMP levels. Furthermore, it could be demonstrated, that GlmM plays a role in the adaption to hypoosmotic shock and that the enzyme is mainly localized in the cytoplasm. A GlmM variant that contained the F154I mutation, which lies in a highly conserved region of the protein, exhibited reduced effects on CdaA compared to the wild type GlmM. This gives a first hint that this site is involved in the interaction of the two proteins. The GlmM protein from *Escherichia coli* did not affect the activity of CdaA from *L. monocytogenes*, hinting at a divergent evolution of this essential enzyme in bacteria that possess a CdaA protein and those who do not. In summary, this work was able to expand the understanding of the *in vivo* regulation of the DAC CdaA and the phosphoglucosamine mutase GlmM.

## 564-SeSiP

PROMIS4Cyanos, a new method to predict and identify new protein-metabolite interactions in Cyanobacteria

\*A. Kraus<sup>1</sup>, E. Sokolowska<sup>2</sup>, D. Schlossarek<sup>2</sup>, A. Skirycz<sup>2</sup>, W. R. Hess<sup>1</sup>

<sup>1</sup>Albert-Ludwigs-University, Institute for Biologie III, Freiburg, Germany

<sup>2</sup>Max Planck Institute of Molecular Plant Physiology, , Potsdam-Golm, Germany

Protein-small molecule interactions can play important roles in the regulation of metabolism and development. In cyanobacteria, some transcription factors bind small metabolites as co-repressors or inducers. The promoter binding of the activator CmpR is stimulated through 2-phosphoglycolate and ribulose 1,5-bisphosphate (Nishimura *et al.*, 2008). For the repressor NdhR(CcmR), the metabolites 2-oxoglutarate and NADP<sup>+</sup> have been confirmed as corepressor molecules (Daley *et al.*, 2012), thereby increasing the binding of this repressor to two regions upstream of *ndhR* and *ndhF3* (Figge *et al.*, 2001). 2-oxoglutarate is also the signaling molecule for NtcA, a regulator of nitrogen homeostasis in cyanobacteria, that regulates the transcription of genes related to nitrogen assimilation. NtcA with its promoter element is not sufficient for the activation of transcription and 2-oxoglutarate is required for transcriptional initiation (Tanigawa *et al.*, 2002). However, due to the lack of system-wide approaches, only a minority of those regulatory molecules are known. In the center of PROMIS (PROtein Metabolite Interactions using Size separation) is a simple biochemical method utilizing the big size difference between proteins and small molecules. The hypothesis is that small molecules/metabolites interact with proteins and form stable complexes which will fractionate together, appearing in the high molecular weight fractions. Our metabolomics based analysis of small molecules co-fractionating with proteins identified a multitude of different metabolites in *Synechocystis* PCC 6803-M, suggesting the existence of small molecules bound to proteins that represent new potential regulatory molecules. The following proteomics based analysis across protein-containing fractions that show similar elution behavior like the analyzed metabolites result in the prediction of new regulatory interaction partners. The method shown here was established for *Arabidopsis thaliana* and was adapted to *Synechocystis* PCC 6803-M cell cultures, but is also applicable to other biological systems (Veyel *et al.*, 2017 and 2018).

## 565-SeSiP

### Going green - Influence of plant root exudates on phenotypic traits of insect pathogenic *Photorhabdus luminescens*

\*N. Dominelli<sup>1</sup>, A. Regaiolo<sup>1</sup>, K. Andresen<sup>1</sup>, R. Heermann<sup>1</sup>

<sup>1</sup>Johannes-Gutenberg-Universität Mainz, Institut für Molekulare Physiologie, Mikrobiologie, Mainz, Germany

*Photorhabdus luminescens* is a bioluminescent, Gram-negative entomopathogenic bacterium of the family of *Enterobacteriaceae*. Its dualistic life cycle involves a mutualistic symbiosis with *Heterorhabditis bacteriophora* nematodes and a pathogenic part towards a wide range of insect hosts. *P. luminescens* exists in two phenotypically different cell variants: primary (1°) and secondary (2°) cells. 2° cells lack several 1° specific features like bioluminescence, pigmentation and antibiotic production. Furthermore, in contrast to 1° cells, they are not able to live in symbiosis with the nematodes anymore and are therefore left in soil when all nutrients of the insect cadaver are depleted. This leads to the assumption that 2° cells are better adapted to a free life in soil, which suggests a direct interaction with or attraction towards plants in the rhizosphere. Here we show the influence of plant deriving signals on *P. luminescens* 2° cells.

RNA-Seq data of 2° cells cultivated with root exudate gave first insights in the effects of plant root deriving substances on the expression of genes related to metabolism, transport, flagellar machinery etc. Since genes related to chitinase activity are upregulated in 2° cells due to plant root exudates, the influence of the bacteria on plant pathogenic fungi growth was analysed and characterized. Our data clearly show that 2° cells have an alternative lifestyle than 1° cells, and utilize alternative nutrients, which are supposed to be plant derived. Moreover, our data show that 2° cells can colonize plant roots and can further act as plant protecting microorganism against plant pathogenic fungi. Since *P. luminescens* is used as bioinsecticide the understanding of how 2° cells can protect plants not only against insects but also against plant pathogenic fungi will improve sustainable pest management in agriculture.

## 566-SeSiP

### Temperature-dependent modulation of the sRNA CyaR in *Yersinia pseudotuberculosis*

\*S. Pienkoß<sup>1</sup>, K. Urban<sup>1</sup>, M. Klöhn<sup>1</sup>, M. Holler<sup>1</sup>, F. Narberhaus<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Microbial Biology, Bochum, Germany

The bacterial regulation of gene expression consists of a diverse and complex regulatory network. In addition to the regulation at DNA level, RNA-mediated regulation represents a mechanism that ensures the rapid adaptation of bacteria upon changing environmental conditions e.g. temperature, osmotic stress and nutrient uptake. Regulatory small non-coding RNAs (sRNAs) are versatile RNA elements that mostly regulate target mRNAs posttranscriptionally by base-pairing in a positive or negative manner. On the one hand mRNAs are stabilized or the binding of the ribosomal subunit is facilitated, on the other hand the sRNA-mRNA-complex leads to the degradation of the mRNA or prevents the binding of the ribosomal subunit (1,2). We focused on the Gram-negative foodborne pathogen *Yersinia pseudotuberculosis* and its temperature-dependent RNA-mediated gene expression under environmental (25 °C) and infection-relevant conditions (37 °C). Based on PARS profiling (1), the sRNA CyaR was identified as an sRNA that undergoes a structural change of the RNA secondary structure in response to an increasing temperature. This led

us to the hypothesis of a temperature-dependent regulation of CyaR target genes. Due to the high homology of CyaR in *Y. pseudotuberculosis* and *E. coli* we also investigated the temperature-dependent regulation of CyaR in *E. coli*. For *Y. pseudotuberculosis*, a CyaR deletion mutant was constructed and *ompX* was identified as the first known mRNA target.

#### References:

- [1] Gottesman S., Storz G. (2010) Cold Spring Harb. Perspect Biol, 10.1101/cshperspect.a003798.
- [2] Gottesman S. (2004) Annu Rev Microbiol 58:303-328.
- [3] Righetti F. et al., (2016) Proc Natl Acad Sci USA 113(26):7237-7242.

#### 567-SeSiP

Biochemical characterization and crystallization of the iron response regulator IscR of *Dinoroseobacter shibae*

\*L. Plötzky<sup>1</sup>, H. Maaß<sup>1</sup>, P. Lukat<sup>2</sup>, D. Jahn<sup>1</sup>, E. Härtig<sup>1</sup>

<sup>1</sup>TU Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Structure and Function of proteins, Braunschweig, Germany

**Introduction:** *Dinoroseobacter shibae* is a member of the *Roseobacter* group of marine bacteria. In its natural habitat, the highly oxygenated sea water, only a minor amount of soluble ferrous iron is available. *D. shibae* possesses a member of the Rrf2-family of transcription factors that shares 42 % identity to *E. coli* IscR. Different to *E. coli* IscR, IscR of *D. shibae* is supposed to coordinate a heme cofactor. Three histidine residues were identified, which might be involved in heme coordination.

**Objectives:** Biochemical characterization, crystallization and functional analysis of IscR.

**Material & Methods:** To identify the role of the histidine residues for the coordination of heme, each of the three residues was changed to an alanine residue via site directed mutagenesis of the corresponding gene. IscR of *D. shibae* and mutant variants were recombinantly produced and purified aerobically. Coordination of heme as a cofactor was monitored by UV/Vis spectroscopy. DNA binding of aerobic purified IscR wildtype and mutant proteins was analyzed using electro mobility shift assays (EMSA). Purified StreptII-tagged IscR with a concentration of 15 mg/mL was used for the crystallization attempts. Gene regulatory effects of IscR regarding to iron availability were analyzed via a *hemB2-lacZ* reporter gene fusion.

**Results:** Purified IscR showed a characteristic absorption maximum of heme at 425 nm. EMSA analyses revealed binding of the IscR protein to the *hemB2* promoter DNA sequence. Measured  $\beta$ -galactosidase activities indicated a repressor function of IscR. First crystallization attempts resulted in IscR crystals and X-ray data extending to a resolution of 1.9 Å were collected and revealed a dimeric structure of IscR. In addition, it was possible to co-crystallize IscR together with the heme cofactor.

**Conclusion:** The IscR homolog of *D. shibae* is able to measure iron availability by binding of a heme cofactor via histidine residues and regulates target genes in response to iron limitation.

#### 568-SeSiP

PAS4-LuxR solos in *Photorhabdus luminescens* as inter-kingdom-signaling receptors

\*J. Brehm<sup>1</sup>, L. Antelo<sup>2</sup>, E. Thines<sup>1,2</sup>, R. Heermann<sup>1</sup>

<sup>1</sup>Johannes Gutenberg-Universität Mainz, Mikrobiologie und Weinforschung, Mainz, Germany

<sup>2</sup>Institut für Biotechnologie und Wirkstoff-Forschung, Kaiserslautern, Germany

The well-known cell-cell-communication systems in Gram-negative bacteria are based on LuxR-type receptors detecting specific signaling molecules to regulate gene expression. These receptors contain a C-terminal DNA binding domain (DBD) and an N-terminal signal binding domain (SBD). The Gram-negative entomopathogen *Photorhabdus luminescens* harbors the remarkably high number of 40 LuxR-type receptors, most of them harbor a "PAS4"-SBD1. Knockouts of *PAS4-luxR* gene clusters showed a specific decrease in pathogenicity against larvae from different insect species. Therefore, the PAS4-LuxR receptors are supposed to play a central role in inter-kingdom-signaling between the bacteria and their eukaryotic hosts. The "PAS3"-SBD of the insect juvenile hormone binding regulator Met of *Drosophila melanogaster* is structurally very homologous to the "PAS4" domains of the LuxR solos Plu2019 and Plu2018. This fact suggested that the Plu2018/Plu2019 "PAS4" domains might also sense insect hormone-like molecules. Among others, *plu0258* was identified as a target gene of Plu2018 and/or Plu2019. *Pplu0258* activity could be specifically induced by *Galleria mellonella* insect homogenate revealing that the signal, which is sensed by Plu2018/Plu2019, is derived from this eukaryotic host. We then isolated the Plu2018/Plu2019 signaling molecule from *G. mellonella* insect homogenate and curtailed the number of possible ligands to a few comparatively hydrophobic small molecules. In a HPLC fraction we identified a molecule, which shows a sterol-like UV spectrum and is putatively sensed by the inter-kingdom-signaling PAS4-LuxR solos. Since PAS4-LuxR solos are also present in human pathogens, they might represent promising targets for novel antimicrobial drugs.

- [1] Brameyer, S., Kresovic, D., Bode, H.B., Heermann, R. (2014). LuxR solos in *Photorhabdus* species, Front Cell Infect Microbiol. 2014; 4:166.

#### 569-SeSiP

Establishing and reversing cell polarity during phototactic movement of cyanobacteria

\*Y. Han<sup>1</sup>, A. Jakob<sup>1</sup>, N. Schuergers<sup>1</sup>, A. Wilde<sup>1</sup>

<sup>1</sup>Institute of biology III, university of Freiburg, Freiburg, Germany

The unicellular cyanobacterium *Synechocystis* sp. PCC 6803 can move towards or away from a unidirectional light source using type IV pili, a process called phototaxis. Different photoreceptors involved in phototaxis have been identified and it was observed that the pilus motor ATPase PilB1 dynamically localizes to the leading edge of the motile cell [1, 2]. However, the molecular mechanisms by which the perceived light signals lead to the polar activity of the motility apparatus are still not well understood. Previous studies suggest that specific CheY-like response regulators (RR) play key roles in establishing light-induced motility and cell polarity in *Synechocystis* [1]. While some contain only a CheY-type receiver domain (REC) others belong to the PatA-type regulators where the REC domain is fused to a – so-far uncharacterized - PATAN domain [1,3].

Using Yeast two-hybrid screens and fluorescence tagging, we aim to uncover the function of these RRs in phototactic orientation.

Our results show that some RRs localize to the cytoplasmic membrane and we confirmed direct protein-protein interactions between the response regulators (PixG and PixE) and the pilus motor protein PilB1 as well as the pilus platform protein PilC in case of the latter. Assays with truncated proteins show that the PATAN domain constitutes the interaction surface.

To better understand the role of this domain, we investigate the interplay of PATAN- and CheY-like domains in PatA-type response regulators by *in vivo* mutant studies and *in vitro* pull-down and co-immunoprecipitation experiments. Moreover, we plan to decipher the structure-function relationship through crystallography.

[1] Wilde, A. & Mullineaux, C. W. (2017). FEMS Microbiol. Rev. 41, 900–922. [2] Schuergers, N., Nürnberg, D. J., Wallner, T., Mullineaux, C. W. & Wilde, A. (2015). Microbiol. (United Kingdom). 161, 960–966. [3] Makarova, K. S., Koonin, E. V., Haselkorn, R. & Galperin, M. Y. (2006). Bioinformatics. 22, 1297–1301.

---

## 570-SeSiP

Killing me slowly

Regulation of biofilm formation in *P. aeruginosa*

\*C. Manner<sup>1</sup>, T. Jaeger<sup>1</sup>, B. Laventie<sup>1</sup>, U. Jenal<sup>1</sup>

<sup>1</sup>University of Basel, Biozentrum, Basel, Switzerland

Bacterial communities called biofilms safeguard bacteria against antibiotics but also effectively protect pathogens from the immune system. *Pseudomonas aeruginosa* biofilms play a major role in chronic lung infections and were linked to poor clinical outcomes. Understanding the exact mechanisms of biofilm formation, maintenance and their role in the human patient is thus of key importance for future treatment options.

Here, we show that a toxin/antitoxin-like module strongly promotes biofilm formation and maintenance in *P. aeruginosa* PAO1.

We found that the proteins interfere with the global second messenger c-di-GMP. Expression of the module is under control of a major signaling pathway regulating virulence and persistence. Importantly, the module is expressed stochastically in the bacterial population, arguing that *P. aeruginosa* has chosen a "bet-hedging" strategy for biofilm formation during infections.

---

## 571-SeSiP

The PAS4-LuxR solo Plu0919 of the insect pathogen

*Photobacterium luminescens* senses a nematode-derived signal

\*M. T. Lechtenfeld<sup>1</sup>, F. Hildebrandt<sup>2</sup>, J. Brehm<sup>1</sup>, R. Heermann<sup>1</sup>

<sup>1</sup>Johannes-Gutenberg-Universität Mainz, Institut für Molekulare Physiologie, Mikrobiologie und Weinforschung, Mainz, Germany

<sup>2</sup>Stockholm University, The Wenner-Gren Institute, Stockholm, Sweden

It is well known that bacteria communicate via small diffusible molecules, a process termed quorum sensing. The basic quorum sensing system of Gram-negative bacteria consists of a LuxI-type signal synthase and a cognate LuxR-

type receptor that detects the respective signal. However, many bacteria harbor so called LuxR solos, which lack a cognate LuxI synthase, so that the signals they sense are mostly unknown. LuxR solos are assumed to play an important role in inter-kingdom-signaling, the communication between bacteria and their eukaryotic hosts. With an amount of 40, the entomopathogenic bacterium *Photobacterium luminescens* harbors the largest number of LuxR solos ever found in bacteria. Most of the *P. luminescens* LuxR solos contain a PAS4 signal binding domain, which are assumed to detect eukaryotic hormone-like signaling molecules<sup>1</sup>. In order to identify the signal for Plu0919, we analysed putative signal cocktails on binding to the purified protein via Nano Differential Scanning Fluorimetry. Hence, we found that *Heterorhabditis bacteriophora* nematode homogenate contained the signaling molecule for this LuxR solo. Microscale Thermophoresis as well as Surface Plasmon Resonance spectroscopy confirmed the signal-dependent binding of Plu0919 to the promoter regions of different Plu0919 target genes. We then analyzed the influence of Plu0919 on the activity of *PantA*, one of the target promoters. *PantA* activity was repressed in the presence of *H. bacteriophora* homogenate. These results indicate that Plu0919 is involved in inter-kingdom signaling of *P. luminescens* to recognize the host and consequently adapting gene expression. Since PAS4-LuxR solos are often involved in regulation of pathogenicity, these class of regulators are promising targets for novel antimicrobials in the future.

[1] Brameyer, S., Kresovic, D., Bode, H.B., Heermann, R. (2014). LuxR solos in *Photobacterium* species, Front Cell Infect Microbiol. 2014; 4:166.

---

## 572-SeSiP

Alternative splicing as an element of signal transduction in multi-step phosphorelay systems in fungi

\*S. K. Bühring<sup>1</sup>, A. Yemelin<sup>1</sup>, K. Andresen<sup>2</sup>, M. Becker<sup>1</sup>, S. Jacob<sup>1</sup>

<sup>1</sup>Institute of Biotechnology and Drug research gGmbH, Kaiserslautern, Germany

<sup>2</sup>Johannes Gutenberg-University Mainz (JGU), Institute of Molecular Physiology, Mainz, Germany

The increase in protein diversity through alternative splicing is widespread in the Metazoa kingdom, but in fungi, it has not been extensively studied so far. In particular for signal transduction processes, such as in multi-step phosphorelay systems, the role of alternative splicing has not yet been scientifically addressed.

Our project focuses on the high osmolarity glycerol (HOG) signaling pathway. In the HOG signaling pathway of *Saccharomyces cerevisiae*, external stimuli are perceived by one single sensor histidine kinase and transmitted by phosphorylation via one single phosphotransfer protein Ypd1p. In contrast to *S. cerevisiae*, many sensor histidine kinases are present in the higher fungi, which can detect several different external stimuli. However, only one single Ypd1p coding gene is present in their genomes. This leads to the question of how different signals can be received as "input" from several sensor proteins in fungi and how different signals can be transmitted independently from one phosphotransfer protein as different "output" signals to various targets. We address this question using the filamentous phytopathogenic fungus *Magnaporthe oryzae*. In preliminary work in *M. oryzae*, we were able to identify different transcripts resulting from one genome sequence of *MoYPD1*, amplified different cDNA variants thereof and found a previously unknown isoform of MoYpd1p at the protein level. This results in our hypothesis that different, possibly signal-specific, isoforms of the protein MoYpd1p are

produced in *M. oryzae* to facilitate a higher variability in signal transduction.

This hypothesis will be comprehensively investigated at the genetic, transcriptional and biochemical level. In addition to complement the "loss of function" mutant  $\Delta$ Moypd1 with different MoYpd1p isoforms, we intend to use proteome and phosphoproteome analyses to characterize the signaling processes and aim to generate "multi-fluorescence-mutants" of the different isoforms.

---

### 573-SeSiP

Cascade regulation of a sulfide oxidation pathway in *Bacillus licheniformis*

\*J. Li<sup>1</sup>, L. Xun<sup>1</sup>, Y. Xia<sup>1</sup>

<sup>1</sup>Shandong University, State Key Laboratory of Microbial Technology, Jinan, China

Hydrogen sulfide at high levels is toxic, inhibiting aerobic respiration, and it can be benefiting at low levels, functioning as a reducing agent and signaling molecule. The dual effects warrant a homeostasis of endogenous sulfide. Sulfide:quinone oxidoreductases (SQR) and persulfide dioxxygenases (PDO) are two key enzymes involved in removing excess sulfide. In *Bacillus licheniformis*, a *sqr-pdo* gene arrangement is preceded *nreBC*, encoding a putative two component regulator. Immediately upstream, several genes for (multidomain) sulfurtransferases, TauE and a thioredoxin (*yrkEFHIJ-ydfQ*) are located and preceded by *yrkD*, encoding a CstR-like transcriptional repressor. 5'-RACE analysis identified transcription initiation sites in front of *yrkD* and *sqr*. Furthermore, we found that YrkD and NreBC form a regulatory cascade. *B. licheniformis* mutants lacking *nreB* and *nreC* lost the ability to oxidize H<sub>2</sub>S while a  $\Delta$ *yrkD* strain transcribed *sqr* without sulfide induction. A gene encoding green fluorescent protein (GFP) was placed behind *pdo* and used as a reporter system. The reporter induction by sulfide was dose dependent. In summary, YrkD functions as a repressor regulating *yrkDEFHIJ-ydfQ-nreBC*, and NreBC acts as an activating system for the expression of *sqr-pdo*. The cascade regulation fine-tunes the expression of the sulfur oxidizing genes in *Bacillus licheniformis*.

---

### 574-SeSiP

Lead-seq: *in vivo* RNA structure probing on a transcriptome-wide scale

\*V. B. Brandenburg<sup>1</sup>, C. Twittenhoff<sup>1</sup>, F. Righetti<sup>1</sup>, A. M. Nuss<sup>2</sup>, A. Mosig<sup>3</sup>, P. Dersch<sup>2,4</sup>, F. Narberhaus<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Biology and Biotechnology, Microbial Biology, Bochum, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Department of Molecular Infection Biology, Braunschweig, Germany

<sup>3</sup>Ruhr-University Bochum, Department of Biophysics, Bochum, Germany

<sup>4</sup>University of Münster, Institute of Infectiology, Center for Molecular Biology of Inflammation, Münster, Germany

The dynamic conformation of RNA molecules is known to have a profound impact on regulatory processes in living cells. This challenges biological research to aim for a pervasive understanding of RNA secondary structures and their implications.

Here, we introduce Lead-seq, a global *in vivo* structure-probing method. This novel method utilizes the ability of lead(II) acetate to induce strand breaks on single stranded RNA sites [1], and combines it with high-throughput sequencing. We applied Lead-seq to elucidate the transcriptome-wide RNA landscape in the enteric pathogen

*Yersinia pseudotuberculosis*. The method provided structural information equally for all four nucleotides. It correctly displayed secondary structures of different types of non-coding RNAs, and revealed global structural features of mRNAs.

The application of Lead-seq to *Y. pseudotuberculosis* cells grown at two different temperatures unveiled the first temperature-responsive *in vivo* RNA structurome of a bacterial pathogen. The identification of temperature-induced local structural rearrangements allowed for detection of thermo-sensing structural features. The translation of candidate genes derived from this approach was confirmed to be temperature regulated. Overall, we established Lead-seq as approach for comparative studies of RNA structures on a global scale.

1. Lindell M et al., RNA. 2002

---

### 575-SeSiP

Polar localizing phosphodiesterase PdeB regulates the motile-to-sessile transition in *Shewanella putrefaciens*

\*V. Kreiling<sup>1</sup>, T. Rick<sup>1</sup>, S. Mielke<sup>1</sup>, F. Rossmann<sup>1</sup>, K. Thormann<sup>1</sup>

<sup>1</sup>JLU Giessen, Giessen, Germany

Bacterial cells maintain a high degree of intracellular organization, which requires the spatial and temporal organization of cellular components. One of the most important ways to obtain this strictly regulated organization is the recruitment of proteins by so-called landmark proteins, such as HubP, which localizes a number of different protein complexes, such as the chemotaxis system, the origin of the chromosome, or components of type IV pili, to the designated cell pole. We recently showed that in *Shewanella putrefaciens* also the phosphodiesterase PdeB localizes in a HubP-dependent manner to the flagellated cell pole by direct interaction of both proteins.

PdeB affects the global level of c-di-GMP within the bacterial cell in dependence of the environmental conditions. However, we show that detaching of PdeB from the cell pole by single residue substitutions results in a drastic phenotype, strongly suggesting that polar recruitment by and/or interaction with HubP is critical for the normal function of PdeB. We also demonstrate that by regulating the level of c-di-GMP, PdeB controls the motile to sessile transition and thereby decreases flagella-mediated spreading in soft agar. By using transcriptional reporter fusions and fluorescence microscopy, we show that PdeB regulates the expression of the secondary lateral but not the main the polar flagellar system. In addition, PdeB controls the reversible and irreversible attachment to surfaces by regulating the assembly of MSHA pili at the functional level and the expression of *bpf* operon, encoding a surface adhesin, which limits motility of the cells in structured environments. Thus, PdeB is a major mediator of the motile-to-sessile transition in *S. putrefaciens*.

---

### 576-SeSiP

The author has not agreed to a publication.

---

### 577-SeSiP

PQS cleaving dioxygenases from *Nocardia farcinica* and *Streptomyces bingchengensis* interfere with *Pseudomonas aeruginosa* quorum sensing

S. Fetzner<sup>1</sup>, \*A. Arranz San Martín<sup>1</sup>

<sup>1</sup>IMMB, WWU Münster, Münster, Germany

## Introduction

*Pseudomonas aeruginosa* is a nosocomial pathogen that regulates its virulence via a complex quorum sensing network, in which the alkylquinolone signal molecule 2-heptyl-3-hydroxy-4(1*H*)-quinolone (*Pseudomonas* quinolone signal, PQS) plays a crucial role together with 2-heptyl-4(1*H*)-quinolone and *N*-acylhomoserine lactones. PQS can be inactivated by ring cleaving dioxygenases such as Hod from *Arthrobacter* sp. Rue61 and Aqdc from *Mycobacteroides abscessus*. Nonetheless, susceptibility to denaturation under adverse physicochemical conditions and proteolytic degradation are major drawbacks of enzymatic inactivation.

## Materials and methods

In this study, a diverse group of PQS cleaving dioxygenases was characterized regarding PQS cleaving ability, stability, and susceptibility to *P. aeruginosa* extracellular proteases. Furthermore, assays on virulence factor production and biofilm formation by *P. aeruginosa* were performed with the most promising candidates, and their effect on *P. aeruginosa* virulence was assessed in a *Galleria mellonella* infection model.

## Results

Under the conditions tested, the dioxygenase from *N. farcinica* showed the highest thermostability and catalytic efficiency towards PQS, whereas the one from *S. bingchenggensis* showed the highest affinity towards this substrate. Extracellular addition of these PQS-dioxygenases to *P. aeruginosa* cultures led to a reduction in alkylquinolone production. Pyocyanin and pyoverdine levels were also substantially reduced upon enzyme addition. Furthermore, treatment with the most promising PQS cleaving dioxygenases showed a slight protective effect when *Galleria mellonella* larvae were infected with *P. aeruginosa* PAO1. Further investigations to analyze the effects of quenching a compound that is both a signal molecule and an iron chelator not only on *P. aeruginosa*, but within the context of polymicrobial infections, would be highly interesting.

## 578-SeSiP

### Membrane Stress induces T6SS activity in *Pseudomonas aeruginosa*

\*A. S. Stolle<sup>1</sup>, B. Meader<sup>2</sup>, J. J. Mekalanos<sup>2</sup>

<sup>1</sup>Westfälische Wilhelms University, Center for Molecular Biology of Inflammation, Münster, Germany

<sup>2</sup>Harvard Medical School, Microbiology and Immunobiology, Boston, United States

The type 6 secretion system (T6SS) is a nanomachine encoded by many gram-negative bacteria used for warfare among different species to create a growth advantage in densely occupied niches. Some species, like *Vibrio cholerae*, use their T6SS in an untargeted fashion and seemingly fire randomly at other bacteria. In contrast, *Pseudomonas aeruginosa* uses its T6SS more defensively, meaning that it only fires its T6SS when it has been challenged before. Interestingly, *P. aeruginosa* seems to sense the exact position of an incoming attack, because it is able to retaliate at the same position of the incoming attack. However, the signal that is sensed by *P. aeruginosa* has not been identified to date. Here, we use a CRISPR interference approach to knock down essential genes in the inner membrane, periplasm or outer membrane to interrogate whether membrane disruption is the signal that is sensed by *P. aeruginosa*. Indeed, knockdown of *bamA*, *tolB* and *lptD*

led to increased functional T6SS activity and results in untargeted killing of T6-negative *V. cholerae*. Assembly and firing of the T6SS apparatus is signaled via the threonine phosphorylation pathway. Here, we show that outer membrane perturbation is sensed by *P. aeruginosa* and results in building of a functional T6SS apparatus.

## 579-SeSiP

The author has not agreed to a publication.

## 580-SeSiP

The author has not agreed to a publication.

## 581-SeSiP

The phosphotransferase protein EIIA<sup>Ntr</sup> regulates AcnB aconitase activity in a phosphorylation-independent manner in *Escherichia coli*

\*F. Sikora<sup>1</sup>, M. Mörk-Mörkenstein<sup>1</sup>, B. Görke<sup>1</sup>

<sup>1</sup>Max Perutz Labs, University of Vienna, Vienna, Austria

Bacteria constantly adapt their metabolism to the availability of carbon and nitrogen sources. An important system for carbohydrate utilization is the canonical PEP-dependent phosphotransferase system (PTS). In parallel, many *Proteobacteria* possess a nitrogen-related PTS, which exclusively exerts regulatory functions and consists of the three proteins EI<sup>Ntr</sup>, NPr and EIIA<sup>Ntr</sup> (encoded by *ptsN*). EIIA<sup>Ntr</sup> regulates various cellular processes, such as phosphate and potassium uptake, depending on its phosphorylation state 1. However, not all phenotypes of *E. coli* lacking *ptsN* can be explained by the known targets of EIIA<sup>Ntr</sup>. For instance, EIIA<sup>Ntr</sup> was observed to stimulate flux through the TCA cycle in a phosphorylation-independent manner, suggesting that the TCA cycle flux is regulated by EIIA<sup>Ntr</sup> levels 2.

In this study, the aconitase AcnB is identified as a novel interaction partner of EIIA<sup>Ntr</sup>. Interestingly this interaction is independent of the EIIA<sup>Ntr</sup> phosphorylation state. Furthermore, absence of EIIA<sup>Ntr</sup>, but not modulation of its phosphorylation, results in higher aconitase activity. This is in contrast to other regulatory targets of EIIA<sup>Ntr</sup>, which interact either with the phosphorylated or the non-phosphorylated form of the protein. These findings suggest that levels of EIIA<sup>Ntr</sup>, whose synthesis is believed to be constitutive, might be modulated by the cell. To identify factors involved in the regulation of *ptsN* expression, we carry out a transposon mutagenesis screen and find that Hfq, a key player in post-transcriptional regulation, negatively impacts EIIA<sup>Ntr</sup> synthesis. In addition, we observe that *ptsN* transcription increases under cell envelope stress conditions. Taken together, we find that *E. coli* can adjust EIIA<sup>Ntr</sup> levels on the transcriptional as well as the post-transcriptional level, allowing to modulate AcnB activity in a phosphorylation-independent manner.

1 Mörk-Mörkenstein, M. et al. *Mol. Microbiol.* 106, 54–73 (2017)

2 Jahn, S. et al. *Biochim. Biophys. Acta - Mol. Cell Res.* 1833, 2879–2889 (2013)

## 582-SeSiP

GMCSF synergizes with LPS for IL-1 $\beta$  secretion through an alternative NLRP3 inflammasome pathway



**Sara Di Carlo, Georg Häcker, Ian Gentle**  
**Institute of Medical Microbiology and Hygiene, Medical**  
**Center–University of Freiburg, Freiburg, Germany**  
\*S. Di Carlo<sup>1</sup>

<sup>1</sup>Institute of medical Microbiology and Hygiene, Freiburg, Germany

Inflammasomes are multiprotein complexes that act as scaffolds for caspase-1 activation, which in turn promotes the activation of the proinflammatory cytokines IL-1 $\beta$  and IL-18 as well as pyroptosis, a form of regulated cell death. There are multiple inflammasomes and among them the NLRP3 inflammasome responds to a wide array of stimuli such as ATP and nigericin, but the actual activation mechanism is still unknown. NLRP3 activation is a two-step mechanism: a first signal called "priming" is required for expression of pro-IL-1 $\beta$  and NLRP3-activation. Then a second "activation signal", triggers the inflammasome complex formation and rapid cytokines secretion through pyroptosis pores. There are however a number of reports showing that NLRP3 can be activated without a second classical stimulus, as ATP and nigericin, where a priming signal such as LPS treatment is enough to trigger a slower and prolonged IL-1 $\beta$  secretion. GM-CSF treatment is one such scenario, whereby GM-CSF synergizes with LPS to promote NLRP3 activation and IL-1  $\beta$  secretion. Moreover, GM-CSF is expressed at high levels at sites of inflammation, as in synovial fluid from patients with rheumatoid arthritis (RA) and anti-GMCSF treatment is in clinical trial for RA and multiple sclerosis.

We investigated the mechanism of GM-CSF-regulated inflammasome activation and IL-1 $\beta$  secretion. We show that GM-CSF synergizes with LPS for IL-1 $\beta$ -secretion in a NLRP3-ASC-caspase 1 dependent manner. GM-CSF/LPS-induced IL-1 $\beta$ -secretion required the necroptosis signaling components RIPK3 as well as RIPK1 kinase activity and MLKL, suggesting a requirement for cell death signaling. However, neither necroptosis nor pyroptosis could be observed during GM-CSF/LPS-induced IL-1 $\beta$ -secretion. We further observed a requirement for mitochondrial reactive oxygen species (ROS), indicating that ROS may be driving NLRP3 activation in response to LPS/GM-CSF. We propose that this pathway represents a natural trigger for NLRP3 activation that is cell death independent and may be relevant for inflammation and inflammatory disease.

### 583-ARP

#### **Characterizing the role of minD2 and minD4 in *Haloferax volcanii***

\*M. Patro<sup>1</sup>, P. Nussbaum<sup>1</sup>, S. V. Albers<sup>1</sup>

<sup>1</sup>University Of Freiburg, Molecular Biology of Archaea, Freiburg imbreisgau, Germany

The process of archaeal cell division has not been studied in detail. However, the extreme halophilic euryarchaeon *Haloferax volcanii*, utilizes the FtsZ-based bacterial-type system (Makarova and Koonin, 2010). It encodes for eight homologs of FtsZ, at least two of which – FtsZ1 and FtsZ2 - are involved in cell division. However, two homologs – CetZ1 and CetZ2 - were shown to play a role in cell morphology (Duggin et al., 2015). In most bacteria, the correct placement of the Z-ring at the center of the cell is ensured by the Min protein system. In *H. volcanii*, four homologs of minD"s were found, but no minC or minE (unlike *E. coli*). Deletion of any of the four MinD homologs and all their possible combinations in *H. volcanii* had no effect on FtsZ1 localization or cell division. However, the minD2 and minD4 mutants showed a clear motility phenotype.

Although the MinD4 deletion mutant was able to assemble archaellum, swimming motility was inhibited which was

similar to known chemotaxis protein deletion mutants. Indeed, the localization of the CheW clusters was altered in this mutant. At closer look, the MinD2 deletion mutant did not only have a motility phenotype, but also showed a much earlier "round" phenotype than the wildtype cells which usually round up only towards the stationary growth phase. As "rounded up" *H. volcanii* cells do not assemble an archaellum, the motility defect is probably a secondary effect of the minD2 deletion. Currently, we are establishing fluorescent fusion proteins to visualize minD2 and are performing pulldown assays to identify interacting protein partners to understand MinD2"s role in cell shape determination in *H. volcanii*.

### 584-ARP

#### **Translational coupling via termination-reinitiation in archaea and bacteria**

\*M. Huber<sup>1</sup>, G. Faure<sup>2</sup>, S. Laass<sup>1</sup>, G. Rizzo<sup>1</sup>, E. Kolbe<sup>1</sup>, C. Wehrheim<sup>1</sup>, K. Seitz<sup>1</sup>, Y. Wolf<sup>2</sup>, E. Koonin<sup>2</sup>, J. Soppa<sup>1</sup>

<sup>1</sup>Goethe University, Institute of Molecular Biosciences, Frankfurt am Main, Germany

<sup>2</sup>NCBI, NLM, NIH, Bethesda, United States

Many genes in archaea and bacteria are organized in operons and are transcribed into polycistronic mRNAs. This enables translational coupling, i.e. translation of a downstream gene depends on translation of an upstream gene. One mechanism of translational coupling is named "Termination-Reinitiation" (TeRe). In this case, ribosomes that terminate translation of an upstream gene remain on the transcript (at least the small subunit), and reinitiate translation at a nearby or overlapping downstream gene.

From 720 genomes of archaea and bacteria, we identified substantial, albeit highly variable, fractions of co-directed overlapping gene pairs in all major prokaryotic phyla. We experimentally tested the predicted coupling in the model archaeon *Haloferax volcanii* and bacterium *Escherichia coli*. To this end, gene overlaps were fused to reporter genes, and variants were compared that did or did not, respectively, allow the ribosome to reach the overlap. Quantification of translational efficiencies revealed that strict translational coupling via TeRe operates at both tested model organisms. All gene pairs contained SD motifs near the 3"-end of the upstream gene. To analyze the role these in translational coupling, the motifs were mutated. The results indicated that the contribution of the SD motif to translational coupling is highly variable, with the SD-less mutants showing 0% to 100% translational efficiency compared to the wild-type genes. Next, the distance between stop codon and start codon was varied, to elucidate the maximal distance a ribosome can scan along the RNA.

Taken together, translational coupling at overlapping gene pairs was experimentally proven for one archaeal and one bacterial species, and the bioinformatics analysis indicates that it might be very widespread in many groups of prokaryotes.

### 585-ARP

#### **Single-domain zinc finger $\mu$ -proteins in *Haloferax volcanii***

\*S. Zahn<sup>1</sup>, C. Nagel<sup>1</sup>, A. Machulla<sup>1</sup>, J. Soppa<sup>1</sup>

<sup>1</sup>Goethe Universität Frankfurt am Main, Institut für Molekulare Biowissenschaften, Frankfurt a. M., Germany

Characterization of the "low molecular weight proteome" of *Halobacterium salinarum* led to the identification of 380



proteins of less than 20 kDa<sup>1</sup>. Most of them had no assigned function, and 20 contained two CPXCG motifs that were postulated to form a zinc finger. These experimental results also guided the annotation of the *Haloferax volcanii* genome. Currently, *Haloferax volcanii* is annotated to encode 282  $\mu$ -proteins with a length of less than 70 amino acids, only 24 of which have an assigned function. 43 of these  $\mu$ -proteins contain at least two CPXCG motifs and are, therefore, most probably single-domain zinc finger  $\mu$ -proteins.

Until now 18 of the respective genes have been deleted, and 11 deletion mutants differed from the wildtype under at least one condition, e.g. growth capabilities with different carbon/phosphate/nitrogen sources, resistance against UV-/pH/oxidative or osmotic stress, biofilm formation, or motility/chemotaxis. Four deletion mutants showed the same pleiotropic phenotype, i.e. swarming motility was considerably decreased, while biofilm formation, resistance to UV irradiation as well as resistance to osmotic stress were increased.

HVO\_2753 encodes the only  $\mu$ -protein containing four CPXCG motifs and thus two zinc fingers. Its deletion led to a loss of swarming and decrease in biofilm formation. Swarming could be restored by the expression of the native HVO\_2753 gene. In contrast, single amino acid mutants in each CPXCG motif did not enhance swarming, proving that all four motifs are essential. A fluorescence assay experimentally confirmed that HVO\_2753 binds zinc. *In vivo* cross-linking revealed that HVO\_2753 forms a complex with other proteins, and the identification of binding partners is currently under way. Taken together, one domain zinc finger  $\mu$ -proteins fulfill important biological functions in *H. volcanii*.

<sup>1</sup> Klein et al., (2007). J. Proteome Res. 6, 1510–1518.

## 586-ARP

Uptake and degradation of disaccharides in *Haloferax volcanii* and *Haloarcula hispanica*

\*M. Ortjohann<sup>1</sup>, U. Johnsen<sup>1</sup>, P. Schönheit<sup>1</sup>

<sup>1</sup>Christian-Albrechts-Universität zu Kiel, Institut für Allgemeine Mikrobiologie, Kiel, Germany

The degradation pathways of hexoses in halophilic archaea have been studied in detail. E.g. *Hfx. volcanii* degrades glucose via a semi phosphorylated Entner-Doudoroff (spED)-pathway (1) and fructose by a modified Embden-Meyerhof (EM)-pathway. Fructose uptake involves a phosphoenolpyruvate dependent phosphotransferase system (PEP-PTS) that forms fructose-1-phosphate (2). However, the catabolism of disaccharides in haloarchaea has not been analyzed in detail so far.

Here we report analyses of transport, cleavage and degradation of disaccharides in *Hfx. volcanii* and *Har. hispanica*. (I) An ABC transporter composed of a substrate binding protein (SBP), two transmembrane domains and a nucleotide binding domain was identified in *Hfx. volcanii* that is promiscuous for the disaccharides maltose, trehalose, cellobiose, sucrose and isomaltulose. (II) A hydrolase from *Har. hispanica* that belongs to the GH68 family and catalyzes the cleavage of sucrose was characterized. (III) Using deletion mutants of genes encoding the key enzymes glucose dehydrogenase and fructose-1-phosphate kinase we could show that in *Hfx. volcanii* disaccharide-derived glucose and fructose moieties are degraded via the spED- and the modified EM-pathway respectively. (IV) Further we present evidence that in *Hfx. volcanii* the PEP-PTS is involved in phosphorylation of sucrose-derived fructose excluding a role of ketohexokinase that has previously been proposed to

phosphorylate fructose to fructose-1-phosphate in *Haloarcula* species (3).

- (1) Sutter et al (2016): J. Bacteriol. 198:2251-2262
- (2) Pickl A et al (2012): J. Bacteriol. 194:3088-3097
- (3) Rangaswamy and Altekari (1994): J. Bacteriol. 176:5505-5512

## 587-ARP

Metagenomic prediction of altiarchoeotal virus-host dynamics in a high-CO<sub>2</sub> subsurface environment

\*S. Eßer<sup>1</sup>, T. L. V. Bornemann<sup>1</sup>, J. Rahlff<sup>1</sup>, A. J. Probst<sup>1</sup>

<sup>1</sup>University of Duisburg Essen, Group for Aquatic Microbial Ecology, Institute for Environmental Microbiology and Biotechnology, Essen, Germany

## Introduction

*Candidatus* Altiaarchaeum hamiconexum are well known for playing a key role in the carbon cycle in cold, subsurface ecosystems, yet viral interactions with *Ca. A. hamiconexum* and respective virus-host dynamics remain an open question.

## Objectives

To answer this question, we used public datasets encompassing more than 1 Tb of sequence information for identifying putatively viral genomes, whose viruses infect Altiaarchaeota and analyzed the abovementioned virus-host dynamics.

## Methods

The metagenomic datasets (study site: CO<sub>2</sub>-driven, cold-water Crystal Geyser, Utah, USA) spanning a sampling period of 6 years, were used to link predicted viruses to Altiaarchaeota via CRISPR-spacer to protospacer matches. In detail, CRISPR systems of Altiaarchaeota were identified with CRISPRCasFinder<sup>(1)</sup> and spacer were extracted via MetaCRAS<sup>(2)</sup> (clustered at 97% nucleotide identity). Viral genomes were predicted in a polyphasic approach encompassing VirSorter<sup>(3)</sup>, VirFinder<sup>(4)</sup>, VogDB<sup>(5)</sup>, FindCircular<sup>(6)</sup> and inhouse scripts.

## Results

Two CRISPR systems with different repeat sequences were identified within the Altiaarchaeota genomes with a total of 491 and 405,685 spacer clusters, respectively. We were able to assign 64 viral genomes to show infection histories with Altiaarchaeota and another 37, which were likely viruses. The virus-host dynamics of two selected viruses were resolved showing a co-correlation with the host and a fully independent pattern, respectively.

## Conclusion

Altiaarchaeota carry highly heterogeneous CRISPR systems showing a great micro-diversity of strains and complex infection histories and dynamics with viruses. The infection followed by cell lysis of Altiaarchaeota likely jump-starts heterotrophic carbon cycling in these subsurface ecosystems.

## References

1. D. Couvin *et al.*, *Nucleic Acids Res*, **46**, (2018).
2. A. G. Moller *et al.*, *PeerJ*, **5:e3788**, (2017).
3. S. Roux *et al.*, *PeerJ*, **3:e985**, (2015).
4. J. Ren *et al.*, *Microbiome*, **5**, (2017).
5. M. Marz *et al.*, *Bioinformatics*, **30**, (2014).
6. A. Crits-Christoph *et al.*, *Environmental Microbiology*, **18:2064-2077**, (2016).

## 588-ARP

Viability of *Methanosaeta* and attached bacteria

\*A. Lonsing<sup>1</sup>, G. Martens<sup>1</sup>, J. Kizina<sup>1</sup>, J. Harder<sup>1</sup>

<sup>1</sup>Max-Planck-Institut für marine Mikrobiologie, Dept. of Molecular Ecology, Bremen, Germany

### Introduction:

*Methanosaeta* spp., a genus of *Archaea*, is one of the predominant methane producers detected in anoxic habitats all over the world. It dominates the methanogenesis from acetate in slowly growing enrichment cultures and wastewater treatment plants. In limonene-degrading methanogenic communities (1) small coccoid bacteria assigned as strain OP3 LiM were found attached to the *Methanosaeta* filaments. The mode of interaction between the cells is undisclosed.

### Objectives:

Live/Dead stains were used to characterize the viability of the microbial community.

### Results:

Here we report the staining of *Methanosaeta* filaments using stains for DNA (DAPI), ribosomal RNA (FISH probe) and lipids (Nile Red). Within the *Methanosaeta* filaments which were observed in phase contrast microscopy, cells without staining by the FISH probe and DAPI were observed. In these cases, Nile Red revealed the presence of the membrane. Viability stainings are currently pursued and will be reported in this contribution.

### Conclusion:

The lack of staining suggested that within the filaments of *Methanosaeta*, cells are present that lack their cytosolic content. Possible biological reasons include viral infections and predatory bacteria.

1. A.-E. Rotaru, R. Schauer, C. Probian, M. Mussmann and J. Harder (2012) Visualization of candidate division OP3 cocci in limonene degrading methanogenic cultures. *Journal of Microbiology and Biotechnology* **22**, 457–461. DOI: 10.4014/jmb.1110.10055

## 589-ARP

### Interaction of Haloarchaeal Gas Vesicle Proteins Determined by Split-GFP

\*A. Jost<sup>1</sup>, K. Völkner<sup>1</sup>, J. Born<sup>1</sup>, F. Pfeifer<sup>1</sup>

<sup>1</sup>Technische Universität Darmstadt, Microbiology & Archaea, Darmstadt, Germany

### Introduction

Split-GFP is an established method to verify protein-protein-interactions (PPI) in bacteria and yeasts *in vivo* and was recently adapted to Haloarchaea [1]. The green fluorescent protein (GFP) is splitted into 2 fragments, NGFP and CGFP that are fused to two proteins of interest. In the case of an interaction, the GFP portions are able to assemble a fluorescent GFP. We used this method to investigate PPI of gas vesicle proteins in *Haloferax volcanii* to unravel gas vesicle formation.

### Objectives

PPI was used to study the interaction of gas vesicle (GV) accessory proteins to gain insights into early stages of gas vesicle formation.

### Methods

The salt-adapted mGFP2 was splitted between the residues 157 and 158 in two fragments, NGFP and CGFP. Each fragment was fused N- or C-terminally to the two GV proteins of interest and the fluorescence of the resulting *Hfx. volcanii* transformants was analyzed.

### Results

The eight accessory proteins GvpF through GvpM were tested and their interactions were determined. Each protein had a major interaction partner except for GvpL, that interacted with five of these proteins, and GvpF contacted GvpL and GvpA. *Vice versa*, GvpA bound GvpF only. Further analysis with fragments of GvpA determined the N-terminal portion of GvpA, and especially the  $\alpha$ -helix 1, as the interaction site for GvpF.

### Conclusion

Split-GFP is a valuable method to detect PPI under high salt conditions *in vivo*. We uncovered interactions between all accessory gas vesicle proteins and determined GvpF as the only interaction partner for the major gas vesicle protein GvpA. Our results imply the existence of a nucleation complex, but further research is required. Interactions will be specified by further fragmentation and point mutations in these proteins.

[1] Winter K., Born J. and Pfeifer F. (2018). *Front. Microbiol.* **9:1897**. doi: 10.3389/fmicb.2018.01897

## 590-ARP

Establishment of a novel haloarchaeal virus-host system

\*S. Schwarzer<sup>1</sup>, H. M. Oksanen<sup>2</sup>, T. E. F. Quax<sup>1</sup>

<sup>1</sup>University of Freiburg, Archaeal virus-host interactions, Faculty of Biology, Freiburg, Germany

<sup>2</sup>University of Helsinki, Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, Helsinki, Finland

Archaeal viruses are known for their high diversity and complex morphologies. However, relatively little is known about their infection strategies. Archaeal viruses face different challenges during viral entry and egress, compared to their bacterial counterparts, as the archaeal cell envelope and its appendages fundamentally differ from those of bacteria. For only a few archaeal viruses, the entry and egress mechanisms have been studied, so far. To investigate the main principles underlying infection strategies of archaeal viruses, we aimed to establish a genetically accessible haloarchaeal virus-host system.

A culture collection screen uncovered several haloarchaeal strains that appeared to be auspicious host organisms to perform virus-host interaction studies. For these strains, optimal conditions for growth and motility were determined. The characterization of these strains revealed that a recently isolated *Haloferax* sp. is a promising organism to study the entry mechanisms of haloarchaeal viruses. This strain is infected by an icosahedral tailed dsDNA virus with a long non-contractile tail (siphovirus morphotype). *Haloferax* tailed virus 1 (HFTV1) is the first known virus infecting a *Haloferax* strain and was isolated together with its host from saline Lake Retba (Senegal) in 2011 (Mizuno et al., 2019).

Currently, we are developing a system to create gene knockouts for *Haloferax* sp. that hosts HFTV1. Furthermore, sequencing and genome annotation of this strain unraveled a close relationship to other members of the genus *Haloferax*, especially to *Hfx. volcanii*. We therefore aim to test if the extensive genetic toolbox developed for *Hfx. volcanii* facilitates experimental analysis of this virus-host system and will help to identify the infection strategies of haloarchaeal viruses.

### 591-ARP

Investigation of the catalytic mechanism of the Heme-Synthase AhbD

\*I. Fix<sup>1</sup>, G. Layer<sup>1</sup>

<sup>1</sup>University of Freiburg, Pharmaceutical Biology and Biotechnology, Freiburg, Germany

Tetrapyrroles, which are also called the "colors of life", play an important role in all three kingdoms of life. The cyclic tetrapyrrole heme is an essential prosthetic group for almost all organisms on earth. The biosynthesis of heme starts with 5-aminolevulinic acid, which is stepwise converted into uroporphyrinogen III. In *Archaea*, the so-called siroheme-dependent pathway is used to transform uroporphyrinogen III into heme.[1]

The last step of the siroheme-dependent route is the conversion of iron-coproporphyrin III to heme, which is catalyzed by the heme synthase AhbD. This enzyme belongs to the Radical SAM superfamily and contains at least two [4Fe-4S] clusters.[2] AhbD catalyzes the oxidative decarboxylation of two propionate side chains of iron-coproporphyrin III to the corresponding vinyl groups of heme by a radical mechanism. For this, the N-terminal [4Fe-4S] cluster is required for the binding of S-adenosyl-methionine and radical formation. The function of the C-terminal cluster is not known. Determination of the iron and sulfide content of the recombinant AhbD from *Methanosarcina barkeri* indicates the presence of a third [4Fe-4S] cluster. To investigate this hypothesis and the function of the additional clusters, different single cluster variants of AhbD from *M. barkeri* were created. Using an *in vitro* enzyme activity assay it was demonstrated, that the C-terminal cluster might be not required for the conversion of iron-coproporphyrin III into heme. In contrast the putative third [4Fe-4S] cluster might be required for heme formation.

### References:

[1] H. A. Dailey, T. A. Dailey, S. Gerdes, D. Jahn, M. Jahn, M. R. O'Brian, M. J. Warren, *Microbiology and molecular biology reviews* : **MMBR** **2017**, 81.

[2] M. Kühner, P. Schweyen, M. Hoffmann, J. V. Ramos, E. J. Reijerse, W. Lubitz, M. Bröring, G. Layer, *Chem. Sci.* **2016**, 7, 4633.

### 592-ARP

Functional characterization of a cobalamin-dependent Radical SAM methyltransferase

\*J. Gagsteiger<sup>1</sup>, G. Layer<sup>1</sup>

<sup>1</sup>University of Freiburg, Institute of Pharmaceutical Science, Freiburg i. Br., Germany

Radical S-adenosyl-L-methionine (SAM) methyltransferases (MT) are known to methylate a broad range of substrates. They belong to the Radical SAM superfamily using a [4Fe-4S] cluster and SAM in order to initiate radical catalysis [1]. A predicted Radical SAM methyltransferase may also be responsible for the methylation of the unreactive sp<sup>3</sup>-hybridized C2 atom of a glutamine residue in methyl-coenzyme M reductase (MCR) of methanogenic archaea. The gene encoding the suspected MT is often located in close vicinity to the *mcr* genes and next to the gene encoding a Radical SAM enzyme responsible for the C5-methylation of an arginine residue, another modification of MCR [2,3]. Besides the Radical SAM domain, amino acid analysis revealed an N-terminal cobalamin-binding domain. Therefore, the enzyme most likely uses methyl-cobalamin as an additional cofactor.

The aim of the project is the *in vitro* characterization of the predicted cobalamin-dependent MT (B12-MT). However, the production and purification of the B12-MT proved to be difficult largely due to solubility issues during heterologous protein production. Therefore, we constructed expression vectors for B12-MT genes from different organisms using vectors encoding different purification and solubility tags. Different production strains and culture conditions were also tested. Recently, we were able to produce the predicted B12-MT from *Methanoculleus thermophilus* in soluble form in *Escherichia coli*. Next, we will establish an enzyme activity assay using different peptides as substrates and, further, we will investigate the role of the cofactors during catalysis.

[1] H.J. Sofia *et al.*, *Nucleic Acids Res.* **2001**, 5, 1097.

[2] J. Kahnt *et al.*, *FEBS J.* **2007**, 18, 4913.

[3] D. Deobald *et al.*, *Sci. Rep.* **2018**, 1, 7404.

### 593-ARP

Characterization of a Radical SAM methyltransferase involved in the posttranslational modification of methyl-coenzyme M reductase

\*R. Hanna<sup>1</sup>, G. Layer<sup>1</sup>

<sup>1</sup>Universität Freiburg, Institut für Pharmazeutische Wissenschaften, Freiburg, Germany

Methyl-coenzyme M reductase (MCR) is an enzyme involved in the last, methane releasing step of methanogenesis in methanogenic archaea. Near the active site, MCR carries several unusual posttranslational modifications of amino acids, one of which is a 5-methylarginine. It is proposed that this modification increases the stability of MCR, but is not crucial overall for enzyme function [1]. The gene *ma4551* from *Methanosarcina acetivorans* C2A is located near the *mcr* gene cluster and codes for a Radical S-adenosyl-L-methionine (SAM) methyltransferase, which is responsible for the methylation of arginine [1]. Although Ma4551 does not show the typical characteristics of cobalamin-dependent Radical SAM methyltransferases, it requires cobalamin as a cofactor/cosubstrate [2]. Ma4551 most likely contains two [4Fe-4S]-clusters, coordinated by conserved three- or four-cysteine motifs. While one cluster is responsible for the

homolytic cleavage of SAM, the function of the second cluster yet remains unknown.

This work aims to establish an *in vitro* enzyme activity assay for Ma4551, using synthetic peptides as substrate. We then aim to determine the minimal peptide length required for substrate binding and want to identify the amino acid residues needed for substrate recognition. Also the function of the second [4Fe-4S]-cluster and the mechanism of cobalamin regeneration need to be investigated.

So far, we were able to produce and purify wild type Ma4551, as well as two cluster depleted variants, each missing one of the Fe-S-cluster. For the wild type enzyme complete enzyme activity was observed when cobalamin was added. Using HPLC analysis, increasing amounts of 5'-deoxyadenosine as product of the radical cleavage of SAM and an increase in S-adenosyl-L-homocysteine concentration as product of the methyl group transfer were measured over time. Simultaneously, the amount of SAM decreased. The two enzyme variants missing either one of the iron-sulfur clusters showed either no activity or only partial activity.

## References

- [1] D. Deobald, et al., *Sci Rep.* **2018**, 8, 7404.  
[2] M. I. Radle, et al., *J Biol Chem.* **2019**, 294, 11712–11725.

## 594-ARP

### Raising a hidden treasure

\*R. Reichelt<sup>1</sup>, R. Zimmermann<sup>1</sup>, C. Hausmann<sup>1</sup>, F. Grünberger<sup>1</sup>, W. Hausner<sup>1</sup>, H. Huber<sup>1</sup>, D. Grohmann<sup>1</sup>

<sup>1</sup>University of Regensburg, Institute of Microbiology and Archaea Center, Regensburg, Germany

For more than three decades, the Archaea Centre Regensburg represents one of the scientific hearts for the study of extremophilic microorganisms and more specifically for organisms from the third domain of life, the Archaea. During this period, original samples were collected from various extreme habitats from all over the world and subsequently used to enrich, isolate and cultivate new microbial species. Currently, the culture collection at the Archaea Centre comprises more than 1500 strains and enrichment cultures. In addition, we can rely on decades of experience and specialized equipment to cultivate extremophiles at the Archaea Centre Regensburg. While some of the archaeal organisms (e.g. *Pyrococcus furiosus* and the fascinating co-culture of *Ignicoccus hospitalis* and *Nanoarchaeum equitans*) became research objects for many laboratories, the majority of the isolated strains is still not well characterized and awaiting their awakening.

To raise this hidden treasure, we plan to combine state-of-the-art technologies for assaying microbial communities with classical and innovative microbial profiling strategies. This includes e.g. reliable and reproducible protocols for nucleic acid purification and 16S rRNA gene profiling by qPCR and amplicon sequencing. Especially, we are focusing on the implementation of third-generation sequencing platforms as the Nanopore MinION platform in our pipelines.

Here, I will present a case study focusing on four original samples collected at deep-sea hydrothermal sediments and vents in the 80's, 90's, 00's and 10's. So far, we determined microbial community compositions by Nanopore-based sequencing and advantages and disadvantages of this

technique compared to other sequencing platforms will be discussed. Moreover, cultivation attempts showed that even original samples stored at 4°C for more than 30 years can serve as inoculum to successfully isolate microorganisms.

Raising the hidden treasure of the Archaea Centre Regensburg, our ultimate goal is to contribute new experimental strategies to cultivate the unculturable.

## 595-ARP

Polyploidy in prokaryotes: Intermolecular gene conversion as a mechanism to equalize genome copies

\*A. Borst<sup>1</sup>, K. Ludt<sup>1</sup>, M. Hammelmann<sup>1</sup>, J. Soppa<sup>1</sup>

<sup>1</sup>Goethe-Universität Frankfurt, Institut für molekulare Biowissenschaften, Frankfurt a. M., Germany

It has been assumed for many years that monoploidy is a typical feature of prokaryotes. However, in contrast, the majority of prokaryotic species is in fact oligoploid or polyploid. Polyploidy has most likely evolved independently in various phylogenetic lineages and is related to many evolutionary advantages. These include resistance to double strand breaks and the usage of genomic DNA as a phosphate storage polymer. Furthermore, intermolecular gene conversion can lead to the equalization of chromosomes and is defined as a non-reciprocal transfer of information between two homologous DNA sequences.

*Haloferax volcanii* is a polyploid halophilic archaeon and contains about 20-30 copies of its main chromosome when cultivated under optimal growth conditions. We established an approach to study intermolecular gene conversion by fusion of a tryptophan-auxotrophic and a thymine-auxotrophic strain of *Haloferax volcanii* and subsequent selection in the absence of both substrates. Additionally, the strains carry different versions of other genes, which ideally do not underlie any selective pressure and have an easy to identify phenotype. We made use of the genes *crtB* (HVO\_2524) and *crtD* (HVO\_2528), both of which encode an enzyme essential for carotenoid biosynthesis. Strains with different mutations in one or both genes were generated and fused. The efficiency of unselected intermolecular gene conversion was addressed phenotypically and by analytical PCR. This approach will be used to shed light on the importance of different parameters that might influence gene conversion efficiency e.g. 1) the size of insertions/deletions; 2) the size of mismatches; 3) the lengths of DNA-tracts that are converted and 4) the direction of the conversion.

## 596-ARP

Shape and structure of archaeal cells, as seen by electron tomography

\*R. Rachel<sup>1</sup>, L. Dengler<sup>2</sup>, A. Bellack<sup>2</sup>, R. Reichelt<sup>2</sup>, V. Heinz<sup>1</sup>, D. Grohmann<sup>2</sup>

<sup>1</sup>University of Regensburg, Centre for Electron Microscopy, Regensburg, Germany

<sup>2</sup>University of Regensburg, Dept of Microbiology and Archaea Centre, Regensburg, Germany

Various archaeal cells serve as model system for studies of archaeal molecular cell biology. One example is the euryarchaeon *Pyrococcus furiosus*, a motile cell expressing numerous archaeella. Another archaeon studied in our lab is *Methanocaldococcus villosus*, a hyperthermophilic methanogen with exceptional motility. These cells are described as 'slightly irregular' cocci, up to 1500 nm in diameter. They are regarded as round but a detailed analysis is lacking.

**Methods:** Cells are analysed by electron tomographic methods (Rachel et al, 2010). Using electron cryo-tomography and sub-tomogram averaging, we presented a model of the *Pyrococcus furiosus* archaeall motor in 3D (Daum et al. 2017). In current studies, cells are not concentrated by centrifugation, but by gentle filtration, then cryo-immobilized by high-pressure freezing, followed by freeze-substitution and resin embedding. Parallel beam STEM tomography with dual-axis tilting of 600 nm sections enables us to eliminate the focus gradient at high tilt, by increasing the depth of focus. We extend our studies towards naturally occurring lab mutants.

**Results:** The tomography datasets help us to visualize the cells with minimal distortion, using the improved sample preparation protocol. The natural shape of the cells is trapped in an undisturbed way, before their structure is analysed at a resolution of about 5 nm. We unravelled yet undescribed features, like kinks and sharp bends in the archaeal cell wall, features pointing to unique mechanisms of cell shape determination.

**Conclusion:** Combined ultrastructural and biochemical analyses are necessary in order to fully understand the complexity of coccoid archaeal cells. We plan to complement our studies using electron cryo-tomography, in the near future.

#### References:

Daum et al, 2017: eLife 6: 27470

Rachel et al, 2010: Meth Cell Biol 96: 47-69

---

#### 597-PMP

Establishing a hydrogen-sensing platform organism for the directed evolution of continuous, photosynthesis-driven hydrogen production

\*F. Opel<sup>1</sup>, A. Schmid<sup>1</sup>, S. Klähn<sup>1</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Solar Materials, Leipzig, Germany

**Introduction:** Molecular hydrogen (H<sub>2</sub>) is believed to be an ideal candidate for future energy supply because it is a clean, CO<sub>2</sub>-neutral energy carrier. Hence, a biotechnological production of H<sub>2</sub>, i.e. driven by photosynthesis appears promising. Nevertheless, a plethora of hurdles have to be overcome to establish, e.g. a cyanobacterium as whole-cell biocatalyst for a photosynthetic H<sub>2</sub> production.

**Objectives:** We aim to implement the natural H<sub>2</sub>-responsive regulatory cascade of *Ralstonia eutropha*; comprising an O<sub>2</sub>-insensitive H<sub>2</sub>-sensing Hydrogenase, a histidine kinase and a response regulator; in the cyanobacterial model strain *Synechocystis* sp. PCC 6803 (*Synechocystis*). The regulatory components will be coupled with the expression of a reporter gene, namely for the superfolder green fluorescent protein (sfGFP), to achieve a H<sub>2</sub>-dependent sfGFP fluorescence.

**Material & Methods:** The structural and accessory genes encoding the proteins of the regulatory cascade will be combined as artificial operons via PCR-based and gene synthesis approaches for the generation of a series of replicative, broad-host range plasmids for the introduction into *Synechocystis*. In addition, different promoters and ribosome binding sites shall be tested to verify expression as well as function of the respective proteins. The regulatory

cascade will be stepwise established into both *E. coli* and *Synechocystis*.

**Results:** So far, we were able to accomplish a weak transcription regulation mediated by the heterologous response regulator HoxA, visualized by sfGFP in *E. coli*.

**Conclusion:** The completed system would present a synthetic biosensor, which could ultimately be used for *in vivo* monitoring of H<sub>2</sub> and subsequently as tool for the systematic improvement of cyanobacterial H<sub>2</sub> evolution.

---

#### 598-PMP

Elucidating the significance of Sodium for Metabolism in the Cyanobacterium *Synechocystis* sp. PCC 6803

\*M. Burkhardt<sup>1</sup>, K. Forchhammer<sup>1</sup>

<sup>1</sup>Universität Tübingen, Organismic Interactions, Tübingen, Germany

Salts play an important role in life. They serve as buffer substances, cofactors, enhancers and inhibitors and much more. Sodium is the most abundant cation in the environment and is thus expected to influence all living cells. In cyanobacteria, sodium is reported to be required for the regulation of osmotic pressure, pH, bicarbonate uptake and photosynthesis. *Synechocystis* sp. PCC 6803, hereafter *Synechocystis*, is a non-diazotroph unicellular cyanobacterium. If faced with nitrogen starvation, it can enter a state of dormancy termed chlorosis and recover from that in presence of nitrogen again. The goal was to find the primary requirement for sodium in *Synechocystis* and characterise the necessity throughout regular growth and nitrogen starvation-induced chlorosis. Viability and growth were tested with growth curves and measurement of the photosystem 2 activity via PAM fluorometry. Levels of glycogen throughout chlorosis and resuscitation were analysed by enzymatic assay. Nitrite levels during resuscitation were determined by a chemical assay (Griess reaction). Cells in nitrate-supplemented medium died without sodium. The requirement for sodium could be bypassed by addition of 10 mM glucose and gassing with 2% CO<sub>2</sub> but not by addition of 100 mM KHCO<sub>3</sub>. Sodium starvation during early chlorosis led to death of cells, but starvation after glycogen levels were established yielded no difference to regular conditions. Without sodium, cells started to resuscitate but stopped when carbon uptake became relevant. Thus, it was concluded that the activity of the sodium-dependent bicarbonate uptake systems BicA and SbtA are the primary requirement for sodium. This requirement appears omnipresent throughout the life of *Synechocystis*.

Klotz, A., et al, 2016. Awakening of a Dormant Cyanobacterium from Nitrogen Chlorosis Reveals a Genetically Determined Program. Curr. Biol. 26, 2862–2872.

Shibata, M., et al, 2002. Genes Essential to Sodium-dependent Bicarbonate Transport in Cyanobacteria FUNCTION AND PHYLOGENETIC ANALYSIS. J. Biol. Chem. 277, 18658–18664.  
<https://doi.org/10.1074/jbc.M112468200>

---

#### 599-PMP

Investigation of phasins for an increased PHB yield in *Synechocystis* sp. PCC 6803

\*J. Alford<sup>1</sup>, M. Koch<sup>1</sup>, K. Forchhammer<sup>1</sup>

<sup>1</sup>University of Tuebingen, Interfaculty Institute of Microbiology and Infection Medicine Tuebingen, Tuebingen, Germany

Due to its material properties and biodegradability the polyester Polyhydroxybutyrate (PHB) represents a promising alternative to conventional petroleum-based plastic. PHB is naturally produced by many organisms, often under conditions of nutrient limitation. One example is the cyanobacterium *Synechocystis* sp. PCC 6803 (*Synechocystis*), which is a natural PHB producer (Klotz et al., 2016). Since this organism can grow phototrophically, it produces bioplastic from CO<sub>2</sub> as the sole carbon source. To increase the PHB production by *Synechocystis*, it is necessary to better understand its PHB metabolism. So far it is known, that PHB accumulates in granules, which are coated with a layer of structural and functional proteins termed phasins (Bresan et al., 2016). In heterotrophic bacteria, various phasins with different roles in PHB metabolism have been identified (Sznajder et al., 2015). In cyanobacteria only one phasin, PhaP, which regulates the surface-to-volume ratio of PHB granules, has been identified so far (Hauf et al., 2015). Here, we aim to identify and characterize further phasins in *Synechocystis*. For this purpose, we are performing proteome analysis of purified PHB granules. Subsequent molecular analysis of putative phasins could provide deeper insights into their physiological function. Currently, we are investigating the function of the putative phasins Slr0058 and Slr0060. Slr0058 shows sequence similarities to PhaF, a phasin that ensures equal distribution of PHB to the daughter cells during cell division (Maestro and Sanz, 2017). We observed, that a deletion of *slr0058* leads to a reduction in PHB production and to a higher number of PHB granules. We plan to further investigate its function by analyzing its structure and by performing pulldown experiments to reveal interacting proteins. Slr0060 is a potential candidate for an intracellular PHB depolymerase in *Synechocystis*, as its sequence predicts similarities to a family of phospholipases/acylesterase.

## 600-PMP

A new mode of B<sub>12</sub>-remodeling in microalgae?

\*C. Baum<sup>1</sup>, R. C. Menezes<sup>2</sup>, A. Svatoš<sup>2</sup>, T. Schubert<sup>1,2</sup>

<sup>1</sup>Institute of Microbiology, Department of Applied and Ecological Microbiology, Jena, Germany

<sup>2</sup>Max Planck Institute for Chemical Ecology, Research Group Mass Spectrometry, Jena, Germany

B<sub>12</sub>-vitamers are essential cofactors of enzymes involved in central metabolic pathways of bacteria, archaea, humans, animals, and microalgae, but are only produced by selected prokaryotes. The availability of exogenous B<sub>12</sub> directly influences the chance of survival for many B<sub>12</sub>-auxotrophic organisms. In addition, the structural variability among natural B<sub>12</sub>-compounds can limit the fitness of a given B<sub>12</sub>-dependent organism that is specialized on the utilization of a distinct B<sub>12</sub>-vitamer. To circumvent such incompatibilities, selected bacteria (*Dehalococcoides mccartyi*, *Rhodobacter sphaeroides*) and archaea (*Methanosarcina mazei*) possess an enzymatic machinery that allows for structural remodeling of B<sub>12</sub>-vitamers taken up from the environment. In this study, the freshwater model microalga *Chlamydomonas reinhardtii* was investigated for its ability to remodel B<sub>12</sub>-compounds. The organism was fed with structurally different B<sub>12</sub>-vitamers and building blocks of the B<sub>12</sub>-nucleotide loop substructure. The growth of *C. reinhardtii* B<sub>12</sub>-dependent mutant strain was determined via a photometer at a wavelength of 730nm and the remodeling of the nucleotide loop of *C. reinhardtii* was analyzed by B<sub>12</sub>-extraction, purification, and product identification via mass spectrometry. As a result of this study it could be shown that *C. reinhardtii* utilize almost all B<sub>12</sub>-vitamers which were tested but it prefers certain B<sub>12</sub>-vitamers more than others. HPLC and mass spectrometry analyses of B<sub>12</sub>-vitamers extracted from *C. reinhardtii* fed with a not preferred B<sub>12</sub>-vitamer and the building blocks of a preferred

one have shown a shift from the not preferred to the preferred B<sub>12</sub>-vitamer. Based on the collected data a novel mode of biological B<sub>12</sub>-deconstruction in *C. reinhardtii* has to be assumed, the mechanistic basis of which is currently under investigation.

## 601-PMP

Cyanobacteria as a new host for plant terpene biosynthesis

\*M. Dietsch<sup>1</sup>, I. M. Axmann<sup>2</sup>

<sup>1</sup>Heinrich Heine Universität, Synthetische Mikrobiologie, Düsseldorf, Germany

<sup>2</sup>Heinrich-Heine-University Dueseldorf, Synthetic Microbiology, Dueseldorf, Germany

The topology of the cyanobacterial nucleoid is highly regulated by various levels of negative supercoiling. Depending on external stimuli such as variations in temperature, osmolarity, pH or carbon source, but also the intracellular energy level, cells can quickly adapt and globally modify gene regulation by changing the levels of DNA superhelicity. In turn, supercoiling-sensitive promoters can dynamically be activated or repressed depending on the current energy state of the cell. Our overall aim is to reprogram *Synechocystis* in order to precisely fine-tune its growth rate up to a total growth stop and use Cyanobacteria as promising alternative candidates for the production of a broad class of terpenes. Plant triterpenes form one of the most diverse groups of natural products which include versatile bioactive metabolites. In this work, we link the intrinsic isoprene metabolism of *Synechocystis* to heterologous pathway modules, such as codon-optimized genes for Nootkatone production.

## 602-PMP

Metabolic regulation of isoenzymes in the triosephosphate hub of *Synechocystis* sp. PCC 6803

\*S. Lucius<sup>1</sup>, M. Hagemann<sup>1</sup>

<sup>1</sup>Rostock University, Plant Physiology, Rostock, Germany

The research consortium SCyCode (Switch in Cyanobacteria: Coherent decision-making) is a newly established interdisciplinary research network comprising several working groups focusing on cyanobacterial research. The consortium aims to better understand how cyanobacteria can switch between photoautotrophic and heterotrophic modes of metabolism, in adaptation to internal and external signals. This process demands multi-layered regulations of central metabolic routes in a unicellular system and despite the knowledge of the main pathways, the fine-tune regulation is still largely unknown.

As part of this joint project, our group at the University of Rostock focuses on the regulation of carbon metabolism in *Synechocystis* sp. PCC 6803 under different CO<sub>2</sub> availability. Here we are working on mutants deficient in the isoenzymes GAPDH1/2 (Glyceraldehyde 3-phosphate dehydrogenase) and PGAM1/2 (Phosphoglycerate mutase) from the triosephosphate hub. Metabolic changes after shifts from high CO<sub>2</sub> to ambient air in the light resemble the switch from autotrophic to heterotrophic modes after light and dark changes. Growth experiments of the mentioned mutants in shifting CO<sub>2</sub> conditions combined with metabolomic analysis by LC-MS and of glycogen contents will possibly further elucidate the role of these enzymes in the export of carbon during CO<sub>2</sub> acclimation.

Furthermore, we are aiming to identify carbon metabolism related metabolites and candidate peptides that might have

an influence on the activity of our isoenzymes by overexpressing them heterologously in *E. coli* and performing enzymatic assays *in vitro*.

---

### 603-PMP

The role of serine/threonine-kinases in the metabolic regulation of carbon metabolism in *Synechocystis* sp. PCC 6803

\*T. Barske<sup>1</sup>, M. Hagemann<sup>1</sup>

<sup>1</sup>Universitaet Rostock, Pflanzenphysiologie, Rostock, Germany

As part of the newly established interdisciplinary research consortium SCyCode (Switch in Cyanobacteria: Coherent decision-making) this project is aimed to elucidate a better understanding of regulatory switches between photoautotrophic and heterotrophic modes of metabolism in acclimation to internal and external signals in the model organism *Synechocystis* sp. PCC 6803. We hypothesized that different isoenzymes and protein phosphorylation of key enzymes might play a central role for the allocation of organic carbon into different pathways in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803.

In recent phosphoproteome studies it has been shown that many enzymes of the primary carbon metabolism (e.g. Gap2, Eda, Gnd, Tkt and Eno) were phosphorylated (Mikkat et al. 2014, Spät et al. 2015). However, it is still largely unknown if protein phosphorylation has an impact on protein activities and which protein kinases are responsible for protein modifications. To identify serine/threonine kinases involved in the phosphorylation of enzymes of primary carbon metabolism, a knock-out mutant library of all (putative) 12 annotated serine/threonine kinases (*spkA-L*) was established. The acclimation of the different kinase mutants to fluctuating inorganic carbon conditions and the impact on the phosphoproteome will be analyzed in a comparative study of the 12 annotated serine/threonine kinase mutants. Subsequently, the regulation of certain kinases will be analyzed to elucidate the complex carbon regulatory network in *Synechocystis*.

Mikkat, S., Fulda, S., & Hagemann, M. (2014). A 2D gel electrophoresis-based snapshot of the phosphoproteome in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Microbiology (United Kingdom)*, 160(PART 2), 296–306. <https://doi.org/10.1099/mic.0.074443-0>

Spät, P., Macek, B., & Forchhammer, K. (2015). Phosphoproteome of the cyanobacterium *Synechocystis* sp. PCC 6803 and its dynamics during nitrogen starvation. *Frontiers in Microbiology*, 6(MAR). <https://doi.org/10.3389/fmicb.2015.00248>

---

### 604-PMP

Investigation into Cyanobacterial Iron Uptake in a Simulated Archean Ocean

\*T. C. Enzengmüller-Bleyl<sup>1</sup>, A. J. Herrmann<sup>1</sup>, M. M. Gehringer<sup>1</sup>

<sup>1</sup>Technische Universität Kaiserslautern, Microbiology, Kaiserslautern, Germany

The availability of iron, particularly in modern oceans, affects the growth of living organisms. Cyanobacteria, essential primary producers in both terrestrial and marine environments, require iron to build and maintain their photosynthetic machinery. Prior to the Great Oxygenation Event (GOE), iron was abundantly available as soluble Fe<sup>2+</sup> in the Archean ocean, with concentrations ranging from 40 to 120 µM. The oxygenic activity of cyanobacteria may have led

to the precipitation of Fe<sup>3+</sup> due to oxidation of Fe<sup>2+</sup>, thereby removing iron from the system. As Fe<sup>2+</sup> became scarce, marine cyanobacteria would have needed to adapt to lower iron availability.

Our goal is to investigate the regulation of Fe<sup>2+</sup>/Fe<sup>3+</sup> uptake mechanisms in the marine oxygenic phototrophs *Pseudanabaena* PCC7367 and *Synechococcus* PCC7336.

Essential iron uptake transporters and regulators were identified using *in silico* analysis. Alignments were constructed in MUSCLE (Multiple Sequence Comparison by Log-Expectation) and used to generate gene phylogenies using MEGA (maximum likelihood, 1000 bootstraps). Primers for quantitative RT-PCR targeted the following iron related genes: *fur* (iron homeostasis), *cFTR1* (iron permease), *ARTO* (iron reduction) and *rpoC1* (reference gene). Expression of these genes were determined in late exponential phase cultures of *Pseudanabaena* PCC7367 and *Synechococcus* PCC7336, in medium containing 120 µM Fe<sup>2+</sup> over 24 hours, under an Archean-like atmosphere.

Both *Pseudanabaena* PCC7367 and *Synechococcus* PCC7336 could grow and survive in a simulated Archean ocean. Expression of the iron uptake genes varied over the 24 hour period depending on the amount of Fe<sup>2+</sup> available for uptake. Gene phylogenies illustrated the relatedness of iron uptake genes in the cyanobacterial lineage and their distribution will be mapped onto the cyanobacterial molecular clock (Sanchez Baracaldo 2017).

The data generated in this study provides greater insight into the evolution and diversity of cyanobacterial iron uptake mechanisms in the lead up to the GOE.

---

### 605-PMP

Regulation of glycogen metabolism in *Synechocystis* sp. PCC 6803

\*N. Neumann<sup>1</sup>, K. Forchhammer<sup>1,2</sup>

<sup>1</sup>University of Tübingen, Microbiology/Organismic interactions, Tübingen, Germany

<sup>2</sup>University of Tübingen, Tübingen, Germany

To cope with nitrogen deplete conditions the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 undergoes a determined genetic program initiating a process called chlorosis (Klotz, Georg et al. 2016). Chlorosis is characterized by depigmentation and an arrest of cell cycle while at the same time storage polymers in the form of glycogen and polyhydroxybutyrate (PHB) are accumulated. This results in a dormant like state where long term survival is provided due to low-level photosynthesis (Görl, Sauer et al. 1998). Recent studies showed that resuscitation from the chlorotic state is fueled by degradation of the previously accumulated glycogen. Several isofunctional enzymes are involved in glycogen synthesis and degradation in *Synechocystis* (Doello, Klotz et al. 2018). The phenotype of respective knockout mutants revealed that despite catalyzing the same reactions and showing comparable activity, chlorosis and resuscitation proceeds successfully only with a specific set of these enzymes. Furthermore, we can show that different forms of glycogen are present in *Synechocystis*. We are currently investigating the roles of these different forms of glycogen and the associated enzymes and examine the sophisticated regulatory network of glycogen synthesis and degradation.

---

### 606-PMP

## Design and development of hydrogen producing cyanobacterial strains

\*S. Lupacchini<sup>1</sup>, J. Toepel<sup>1</sup>, R. Stauder<sup>1</sup>, J. Appel<sup>2</sup>, L. Lauterbach<sup>3</sup>, B. Bühler<sup>1</sup>, A. Schmid<sup>1</sup>

<sup>1</sup>Helmholtz-Centre for Environmental Research - UFZ - Research for the environment, Solar Materials, Leipzig, Germany

<sup>2</sup>Botanical Institute, Christian-Albrechts-University, Kiel, Germany

<sup>3</sup>Institute of Chemistry, Technische Universität Berlin, Berlin, Germany

Cyanobacteria are potential candidates to couple oxygenic photosynthesis to the production of molecular hydrogen (H<sub>2</sub>) using a bidirectional hydrogenase. They are regarded as promising "low-cost" microbial cell factories for a sustainable H<sub>2</sub> production. H<sub>2</sub> constitutes a high-energy fuel that burns cleanly in producing only water as its by-product. One of the major challenges in using native cyanobacterial hydrogenases is their high oxygen sensitivity. For this reason, our aim is to express the soluble oxygen tolerant NiFe-hydrogenase of *Ralstonia eutropha* in *Synechocystis* sp. PCC 6803. The active protein will be evaluated regarding H<sub>2</sub> production and consumption. Recently, the functional expression of the oxygen-tolerant hydrogenase from *R. eutropha* in *Synechocystis* has been achieved. Its characterization and optimization constitute the main focus of the project. We will present data regarding strain design, the evaluation of protein expression, and setups for activity tests. Finally, we will show analytic tools for the quantification of H<sub>2</sub> production and consumption under different growth conditions. Future research objectives include protein engineering, in order to improve the H<sub>2</sub> production capacity of the oxygen tolerant hydrogenase.

## 607-PMP

Antisense RNA asPcrL regulates expression of photosynthesis genes in *Rhodobacter sphaeroides* by promoting RNase III-dependent turn-over of *puf*-mRNA

\*C. M. Reuscher<sup>1</sup>, G. Klug<sup>1</sup>

<sup>1</sup>Institut für Mikro- und Molekularbiologie, Gießen, Germany

The facultative phototrophic model bacterium *Rhodobacter sphaeroides* is known for its metabolic versatility. Under microaerobic conditions it synthesizes intracytoplasmic membranes harbouring the pigment protein complexes needed for anoxygenic photosynthesis. For fast adaption to varying environmental conditions the transcription of genes in the photosynthetic gene clusters is tightly regulated. The *puf* operon comprises genes which encode proteins of the light harvesting complex I (LHI) and the reaction centre (RC). RNA-Seq and Northern blot analysis of transcripts derived from the *puf* operon unveiled that also certain small RNAs are transcribed. Up to date two different *puf* operon associated sRNAs were characterized. The Hfq-dependent sRNA asPcrL was detected antisense to the 5' region of the *pufL* gene extending into the *pufA-pufL* intercistronic region. Northern blot results confirmed the presence of asPcrL (~180 nt) under microaerobic and phototrophic conditions. An artificial increase in the amount of the asPcrL by plasmid driven over-expression led to a reduction in the amount of LHI/RC-complexes and *In vivo* reporter assays showed that asPcrL influences *pufL* in an Hfq-dependent manner. Additionally, we could show that the over-expression also influences the half-life of the polycistronic *puf* mRNA. *In vitro* degradation assays show that *pufL* is degraded via RNase III in an asPcrL-dependent manner. Taken together asPcrL is the second non-coding RNA which is associated with the *puf*-operon and plays an important role in processing and degradation of its target mRNA.

## 608-PMP

**Manipulation of *Synechocystis* sp. PCC 6803 on a whole-genome scale**

\*A. Behle<sup>1</sup>, \*W. Murugathas<sup>1</sup>, M. Dietsch<sup>1</sup>, R. Machne<sup>2</sup>, I. M. Axmann<sup>1</sup>

<sup>1</sup>Institute for Synthetic Microbiology, Duesseldorf, Germany

<sup>2</sup>Institute for Quantitative and Theoretical Biology, Düsseldorf, Germany

Regulation of the topological state of the cyanobacterial chromosome is dependent on different external and internal conditions, including, but not limited to nutrient availability, temperature, pH, or the intracellular energy level. The main enzyme class involved in the mediation of DNA topology is the topoisomerases, which comprise a variety of similar enzymes with distinct functions. Mainly, we are interested in the two opposing players – the gyrase, which is able to actively introduce negative supercoils, and the topoisomerase I, which can remove supercoils, thereby relaxing the DNA.

Using a variety of newly developed genetic tools, we aim to precisely target this global regulatory network by manipulating these enzymes on a transcriptional and translational level. We aim to measure these changes in real time, using modern techniques such as transcriptomics and live-cell imaging.

## 609-PMP

**Regulators of cyanobacterial gene expression**

\*L. Berwanger<sup>1</sup>, \*A. Klopp<sup>1</sup>

<sup>1</sup>Heinrich Heine University, synthetic microbiology, Düsseldorf, Germany

The cyanobacterial gene regulation is orchestrated by a circadian rhythm in which the times of light-induced gene expression are synchronized by circadian KaiABC clock proteins. In contrast to other bacteria, gene expression in cyanobacteria is governed by an additional regulatory element since they are the only group of Gram-negative prokaryotes that are capable of oxidative photosynthesis. One of the central mechanisms of gene expression is transcription. The cyanobacterial transcription machine (TM) differs from the TM of other bacteria, e.g. the central protein of the TM - the DNA-dependent RNA polymerase (RNAP) - has several specific properties in contrast to the typical bacterial RNAP. The binding of RNAP to the promoter regions of the gene to be transcribed requires the reversible association of a sigma factor ( $\sigma$  factor). Recognition and binding of DNA promoter elements are initiated by specific  $\sigma$  factors. Cyanobacteria have a broad pool of different  $\sigma$  factors that regulate gene expression depending on specific metabolic states or stresses. In this work, we provide approaches to characterize components that are involved in cyanobacterial gene regulation and expression.

## 610-PMP

**Vitality determination of terrestrial cyanobacteria by means of their oxygen production**

\*M. Witthohn<sup>1</sup>, R. Ulber<sup>2</sup>, K. Muffler<sup>1</sup>

<sup>1</sup>University of Applied Sciences, Life Sciences and Bioengineering, Bingen am Rhein, Germany

<sup>2</sup>Technical University, Bioprocess Engineering, Kaiserslautern, Germany

Recent studies indicate terrestrial cyanobacteria as a great reservoir of secondary metabolites with antifungal, antiviral or antibacterial effects. However, standard microbiologic methods are commonly not applicable, or not easily



adaptable to these bacteria. As an example, cyanobacteria are usually sustained over years by serial transfer on solid agar plates or liquid cultures (Day, 2017), because cryopreservation methods are not sufficiently established. This kind of strain collection causes a high workload and favours contaminations as well as genetic alterations (Rhodes et al., 2006). For the evaluation of new conservation techniques, the determination of cell vitalities is crucial. However, commonly used methods like the counting of cell-forming-units, or the analysis of growth rates are not feasible for many cyanobacteria, due to the formation of cell aggregates, the production of viscous exopolysaccharides, or slow growth rates. To overcome these problems, a new method for the vitality determination of cyanobacteria was developed in this work. An illuminated and tempered setup with an integrated pO<sub>2</sub> electrode was inoculated with formerly cryopreserved and defrosted samples of the terrestrial cyanobacterium *Trichocoleus sociatus*. As cryoprotectants, either DMSO (5% v/v), methanol (5% v/v) or glycerol (15% v/v) was applied. An increase of pO<sub>2</sub> could already be detected after 10-20 min. Cell vitalities were gained by calculation of the highest gradient of the pO<sub>2</sub> curve. Values obtained from fresh cell material served as reference. The obtained results were validated by growth experiments. The methanol samples showed a diminished vitality of 0-18 %, the ones cryopreserved with either DMSO or glycerol reached decent activity values of 80-96% and 60-70%. The presented approach constitutes a simple and quick method for the vitality determination of cyanobacteria and can be adapted for many different scientific issues, as the testing for antibiotic susceptibilities/resistances, or the response to altered cultivation conditions.

## 611-PMP

RNase E Substrate Affinity in the Cyanobacterium *Synechocystis* sp. PCC 6803

\*U. Hoffmann<sup>1</sup>, S. Rogh<sup>1</sup>, F. Heyl<sup>2</sup>, C. Steglich<sup>3</sup>, A. Wilde<sup>1</sup>

<sup>1</sup>Institute for Biology III, Molecular Genetics, Freiburg i. Br., Germany

<sup>2</sup>Department of Computer Science, Bioinformatics, Freiburg i. Br., Germany

<sup>3</sup>Institute for Biology III, Genetics and Experimental Bioinformatics, Freiburg i. Br., Germany

RNase E is an essential endoribonuclease in *E. coli*. The enzyme is involved in RNA maturation and degradation. Its C-terminal half acts as the scaffold of the degradosome. Furthermore, it plays an important role in the action of several sRNAs.

Target recognition by RNase E is based on several factors. Mainly, two pathways were characterized. Firstly, RNase E shows a high affinity to 5'-mono-phosphorylated RNAs. Secondly, there is a pathway termed 5'-bypass with which RNAs' secondary structures are recognized independent of their 5'-phosphorylation status.

RNase E homologues with different structural properties were found in many bacteria. However, its target affinity was mainly investigated in the enterobacteria *Salmonella* and *E. coli*. RNase E of our model organism *Synechocystis* sp. PCC 6803 is homologous to the N-terminal half of *E. coli* RNase E.

Our work aims for understanding the role of RNase E and the roles of its different target recognition mechanisms in *Synechocystis*.

To identify RNase E dependent RNA processing sites on a genome-wide level, we performed tagRNA-Seq of strains with either wild type RNase E or RNase E deficient in 5'-

mono-phosphate sensing. tagRNA-Seq is an RNA-Seq protocol to identify processing and transcriptional start sites on a genome-wide level.

Our results show that 5'-mono-phosphate sensing is, as already known from *E. coli*, also important for rRNA and tRNA maturation in cyanobacteria. Moreover, the processing sites of many other transcripts differ between the two strains. This is also reflected in several phenotypic effects observable between the two different strains.

Further experiments will be conducted to clarify the exact mechanisms underlying our observations.

## 612-PMP

Mixed-trophies biofilms for high-cell-density cultivation of *Synechocystis* sp. PCC 6803 in capillary reactors

\*I. Heuschkel<sup>1</sup>, A. Hoschek<sup>1</sup>, A. Schmid<sup>1</sup>, B. Bühler<sup>1</sup>, R. Karande<sup>1</sup>, K. Bühler<sup>1</sup>

<sup>1</sup>UFZ Leipzig, Solar Materials, Leipzig, Germany

In Nature, almost 385 billion tons of CO<sub>2</sub> is fixed annually to reduced organic materials by photosynthesis. This power of photosynthesis will be crucial for the production of value-added chemicals and fuels and reduce future dependency on fossil resources. However, the lack of scalable photobioreactors that provide efficient light transmission, CO<sub>2</sub> supply, and O<sub>2</sub> degassing and thus enable high cell densities (HCD), constitutes a key bottleneck.

Commercialized tubular photobioreactors offer a surface area to volume ratio (SA/V) of over 100 m<sup>2</sup> m<sup>-3</sup> enabling the efficient capturing of incident solar radiation.<sup>1</sup> However, the high energy demand above 2000 W m<sup>-3</sup> necessary for O<sub>2</sub> degassing and CO<sub>2</sub> supply constitutes a major disadvantage of such tubular reactors. It was estimated to excel the (chemical) energy harvested from the sunlight (150 W m<sup>-3</sup>) by a factor of 13-14, prohibiting an economically feasible photo-biocatalytic production of lower-priced compounds.<sup>1</sup>

We introduce capillary biofilm reactors with a high surface to volume ratio (1333 m<sup>2</sup> m<sup>-3</sup>), and thus enhanced light availability, enabling HCDs of photo-autotrophic microorganisms. However, oxygenic photosynthesis leads to O<sub>2</sub> accumulation in such systems, impairing biofilm growth. We combined O<sub>2</sub> producing *Synechocystis* sp. with O<sub>2</sub> respiring *Pseudomonas* sp. using proto-cooperation to achieve HCDs of up to 51.8 g<sub>BDW</sub> L<sup>-1</sup>.<sup>2,3</sup> This concept was coupled to the challenging C-H oxyfunctionalization of cyclohexane to cyclohexanol with a remarkable conversion of >98% and selectivity of 100 % (KA oil). High photoautotrophic biocatalyst concentrations were established and resulted in productivity of 3.76 g<sub>cyclohexanol</sub> m<sup>-2</sup> day<sup>-1</sup> for at least one month.<sup>3</sup> For benchmarking, the presented phototrophic process will be compared with the conventional chemical and heterotrophic process.<sup>2,3</sup>

## References:

1 Posten, C. Eng. life science. 2009

2 Karande R et al., Patent: PCT/EP2018/074746, 2018

3 Hoschek et al., Biores. tech. 2019

## 613-PMP

Phototrophic microorganisms have the potential to function as biocatalyst for the CO<sub>2</sub>-based synthesis of high energy compounds by mainly using water and CO<sub>2</sub> for photosynthesis. By genetic engineering and optimization of growth condition, the cyanobacterium *Synechocystis* sp. PCC 6803 was shown to be capable of producing a variety of valuable and energy-rich carbon compounds as well as H<sub>2</sub> as an energy carrier of the future. H<sub>2</sub> is produced by transferring electrons via metabolites or directly from the photosynthetic electron transport chain to hydrogenases. Most hydrogenases are inactivated by O<sub>2</sub>, limiting H<sub>2</sub> production by oxygenic phototrophic microorganisms. Also the native hydrogenase of *Synechocystis* is highly O<sub>2</sub>-sensitive. To obtain anaerobic conditions, expensive metal- or enzyme-based O<sub>2</sub> scavengers have been used, but their use on large scale is limited. New reactor designs are necessary to efficiently remove excess O<sub>2</sub> and products, control growth, and efficiently supply microbes with substrates (light and CO<sub>2</sub>). Growth of microbes in biofilm reactors led to enormous advantages including self-immobilization, self-repair, high cell density, and, in case of phototrophs, high light availability (Halan et al., 2012; Posten, 2009). Recently, a mixed-species capillary reactor design containing *Synechocystis* and *Pseudomonas* strains was shown not only to reduce O<sub>2</sub>-levels, but also to induce micro- and anaerobic conditions (Hoschek et al., 2019). Starting from this knowledge, mixed trophy biofilms were studied as a tool to improve H<sub>2</sub> production by *Synechocystis*.

- (1) Halan B., Bühler K. and Schmid A. Trends in Biotechnology, 2012, 30(9), 453-465
- (2) Posten C., Engineering in Life Sciences, 2009, 9(3), 165-177
- (3) Hoschek A., Heuschkel I., Schmid A., Bühler B., Karande R.\*, Bühler K. Bioresource Technology, 2019, 282, 171-178

#### 614-PMP

##### **A synthetic consortium containing *Tolypothrix* sp. and *Pseudomonas* sp. in a capillary biofilm reactor**

\*M. Bozan<sup>1</sup>, A. Schmid<sup>1</sup>, N. Musat<sup>2</sup>, M. Schmidt<sup>2</sup>, K. Bühler<sup>1</sup>

<sup>1</sup>Helmholtz - Centre for Environmental Research (UFZ), Solar Materials, Leipzig, Germany

<sup>2</sup>Helmholtz - Centre for Environmental Research (UFZ), Isotope Biogeochemistry, Leipzig, Germany

Biofilms of photoautotrophic organisms hold a huge potential as an alternative reaction format in biotechnology due to the extraordinary cell densities and the remarkable stabilities possible in such systems. In this context capillary reactors are excellent cultivation systems due to their optimal surface area to volume ratio. [1] Here, we set out to establish a novel strain for biofilm based photo biotechnology, the filamentous and nitrogen-fixing cyanobacterium *Tolypothrix* PCC7712. It was co-cultivated with the aerobic heterotrophic *Pseudomonas taiwanensis* VLB120, following the approach of dual-trophies biofilm cultivation as previously reported for *Synechocystis* sp. PCC6803. [1] *Tolypothrix*/*Pseudomonas* mixed cultures were cultivated either with nitrate, or without to promote nitrogen fixation. As control, single species *Tolypothrix* PCC7712 biofilms were also investigated. Chlorophyll a content was measured as a ready out for *Tolypothrix* PCC7712 growth. Dual trophies cultivation was beneficial for *Tolypothrix* PCC7712 biofilm formation. In mixed cultures cultivated with nitrate a chlorophyll a content

of 32.36 ± 5.83 µg/ml was determined, which is about 4 fold higher than for single species *Tolypothrix* sp. biofilms. Strikingly, nitrogen fixation seemed to strongly promote biofilm formation of both, single as well as dual trophies biofilms, as nitrogen-fixing *Tolypothrix* sp. produced about 4 fold more biomass in the capillaries based on chlorophyll a measurements. Furthermore, confocal laser microscopy and scanning electron microscopy were applied to analyze the localization and morphology of the different species cells in the biofilm. Preliminary results showed that *P.taiwanensis* VLB120 creates a conditioning environment for *Tolypothrix* PCC7712 biofilm by boosting initial attachment of the photoautotrophic organism, and causing higher EPS production. Overall, *Tolypothrix* PCC 7712 appears to be a novel candidate for biofilm based biotechnological applications, due to fast and stable biofilm formation and its ability to fix atmospheric carbon and nitrogen.

[1] Bioresource Technology (2019), 282:171-178. Doi: 10.1016/j.biortech.2019.02.093

#### 615-PMP

##### **Carbohydrate analysis of the cyanobacterium *Synechococcus elongatus***

\*N. Thumm<sup>1</sup>, \*G. Fuentes<sup>1</sup>, L. M. Nentwig<sup>1</sup>, I. M. Axmann<sup>1</sup>

<sup>1</sup>Heinrich Heine Universität Düsseldorf, Synthetic Microbiology, Düsseldorf, Germany

Due to their capability to perform oxygenic photosynthesis cyanobacteria positively influence the world's climate. With the increase of the world population the need for renewable energy sources as well as chemicals and therapeutics is increasing whereas a sustainable production of these compounds seems to be desirable. The microorganisms currently used for the biotechnological production of these products have a heterotrophic lifestyle, which means that they themselves need a carbon source. Cyanobacteria are an attractive alternative to traditionally used heterotrophic organisms, as they are using the energy of the sun, carbon dioxide and water from their environment to build carbohydrates while releasing elemental oxygen. A key carbohydrate is cellulose, an unbranched homopolysaccharide consisting of β-1,4-linked glucose molecules. Cellulose is the most abundant biopolymer on earth primarily produced by plants. Interestingly, cellulose is also a component of the cell wall of certain cyanobacteria. Plant chloroplasts arose through endosymbiosis from cyanobacteria. Thus, a cyanobacterial origin of plant cellulose synthase has been suggested (Nobles et al., 2001). We are aiming to identify the role and importance of cellulose in cyanobacteria. We performed a knock-out of the three genes encoding the bacterial cellulose synthase complex in *S. elongatus*, namely *bcsA*, *bcsB* and *bcsC*. The composition of cell walls of wildtype and mutants is analyzed by linkage analysis via gas chromatography and detected by mass spectrometry.

#### 616-SMP

Electronic analogous synthetic genetic circuits in bacteria for coding, decoding and integrating chemical and microgravity signals in a human designed way.

\*S. Bagh<sup>1</sup>, K. Sarkar<sup>1</sup>, D. Bonnerjee<sup>1</sup>, S. Mukhopadhyay<sup>1</sup>

<sup>1</sup>Saha Institute of Nuclear Physics, Biophysics and Structural Genomics, Kolkata, India

Introduction: Processing complex chemical signals by engineered cells in human designed way is a key importance in cellular robotics. Here we demonstrate our recent effort in

creating complex environmental signal processing circuits in living *E.coli*.

**Objective:** We have created electronic analogous a 2-to-4 biochemical digital decoder, a 4-to-2-priority encoder, 2-to-1 de-multiplexer, and a multiplexer in a bacterial population connected by chemical wires. Those engineered bacteria sense, process, decode, and encode four small molecules environmental chemical signals and response by producing four different fluorescent proteins according to the truth table behavior of the electronic analogous devices.

**Methods:** We have designed, fabricated and assembled synthetic genetic circuits according to electronic circuit principles of the decoder and encoder circuits.

**Results:** All the component genetic logic gates are created using a library of hybrid promoters and experimentally characterized extensively for digitality, dynamic range and mathematically predictive behavior.

**Microgravity Biosensor:** On the other hand, synthetically engineered microbes have numerous projected applications in space bioengineering. Microgravity is a unique property of space. Biological solutions to space travel must consider microgravity as an important component, which is unknown in the biological worlds on the Earth. Therefore, creating a biochemical way to integrate microgravity as a signal within cellular processes is an important step towards developing a technology platform for space bioengineering. Here we have created the first biological or biochemical or molecular microgravity sensor in *Escherichia coli* applying synthetic gene circuits. The engineered bacteria sense and respond to microgravity by alerting the expression of fluorescent reporter protein. We further demonstrate any gene and promoter can be used. Thus, our design is universal.

**Conclusion:** Our work has significance in microbial robotics, synthetic biology and space bioengineering.

---

## 617-SMP

### Light-mediated control of complex biosynthetic pathways in *Pseudomonas putida*

\*N. L. Bitzenhofer<sup>1</sup>, F. Hilgers<sup>1</sup>, A. Loeschcke<sup>1</sup>, J. Ehlers<sup>1</sup>, J. Gebauer<sup>2</sup>, J. Pietruszka<sup>2,3</sup>, K. E. Jaeger<sup>1,3</sup>, T. Drepper<sup>1</sup>

<sup>1</sup>Institute of Molecular Enzyme Technology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

<sup>2</sup>Institute for Bioorganic Chemistry, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

<sup>3</sup>Institute of Bio- and Geosciences IBG-1, Forschungszentrum Jülich, Jülich, Germany

Optimization of bacterial production processes often requires precise control over targeted metabolic pathways. In the last decades, different approaches have been described to develop such processes in bacteria with the implementation of optogenetic tools as a rapidly emerging approach. To control complex biosynthetic pathways with light, we used genetically encoded photosensitizers (PS). These proteins produce reactive oxygen species (ROS) upon illumination and can thus damage cellular components. Here, we report on the development and evaluation of these proteins for chromophore-assisted light inactivation (CALI) of target enzymes in living cells. The feasibility of a light-mediated control over such complex production processes was analyzed by using the bifurcated pathway of the antibiotic tripyrrole prodigiosin as a proof of concept. In initial experiments, we were able to demonstrate a gradual PS-mediated inhibition of the enzyme PigC which catalyzes the final product-forming condensation reaction of the

monopyrrol MAP and the bipyrrrole MBC. The light-triggered PigC inhibition was analyzed by photometric quantification of prodigiosin revealing a significantly reduced enzyme activity upon illumination. Furthermore, a PS-based strategy was developed to enable targeted control over the prodigiosin precursor supply. As observed for PigC, the production level of the precursor MAP and the subsequent formation of prodigiosin could be controlled via light-mediated reduction of the respective enzyme activity. In summary, our results indicate that complex metabolic pathways can principally be light-regulated by using genetically encoded PSs as tags for CALI of defined enzymes. The degree of inactivation, however, depends on the illumination conditions and the applied photosensitizer as well as the targeted enzyme.

---

## 618-SMP

### Design and construction of a new yeast endosymbiont

\*N. T. Odematt<sup>1</sup>, P. D. Gerlinger<sup>1</sup>, T. J. Erb<sup>1</sup>

<sup>1</sup>MPI for Terrestrial Microbiology, Biochemistry and Synthetic Metabolism, Marburg, Germany

Endosymbiosis represents the key step in early evolution, where a metabolically limited eukaryote took up bacteria and transformed them into organelles. This allowed the host to increase their metabolic diversity and occupy new niches. Still, stably inheritable internal bacteria in nature are rare, their understanding limited and manipulation difficult. Artificial endosymbionts can be used as a modular tool to improve bioproduction and exploit the advantages of two fundamentally different organisms.

The generation of a synthetic intracellular symbiont aims at replaying nature by implanting a bacterium into a yeast cell. We tested different mutual dependencies based the exchange of essential vitamins, amino acids or antibiotic protection. This kept *S. cerevisiae* and *E. coli* in close physical association in co-culture experiments, but did not yet lead to an internal propagation of the bacterium.

---

## 619-SMP

### Development of new genetic engineering tools for *Methylobacterium extorquens*

\*L. Pöschel<sup>1</sup>, E. Gehr<sup>1</sup>, P. Jordan<sup>1</sup>, M. Buchhaupt<sup>1</sup>

<sup>1</sup>Dechema Forschungsinstitut, Industrielle Biotechnologie, Frankfurt a. M., Germany

The  $\alpha$ -proteobacterium *Methylobacterium extorquens* is an extensively studied model organism for methylotrophy. It also serves as promising host for the biotechnological production of valuable compounds from C1 carbon sources. The development of efficient production strains usually requires several genetic modifications. The toolbox for genetic engineering of *M. extorquens* is limited. There are only a few promoters established and all plasmids used are based on the same vector system (pCM).

A compatible, stably maintained second plasmid system would allow to test combinations of multiple enzyme activities within a single strain rapidly. In order to find a plasmid that is compatible to the IncP-vector pCM, potential candidates were tested in *M. extorquens* for their functionality. Eventually, we could isolate a mutated version of the pBBR1-based broad host range vector pMis. The respective plasmid pMis1\_1B contains a mutation within the promoter region of the *rep* gene. Although the transformation efficiency and growth rate of pMis1\_1B-transformants is decreased compared to pCM-transformants, it can be used for the

expression of heterologous genes. In subsequent experiments, pMis1\_1B and pCM were successfully tested for compatibility. Surprisingly, reporter gene experiments indicate that expression levels of genes encoded on pMis1\_1B are increased when pCM is present in the same strain.

Furthermore, we optimized the cumate inducible promoter system PQ2148, which showed a leaky repression in our experiments. After expressing a toxic operon under the control of the leaky promoter, we could isolate different suppressor mutants. We found a mutated promoter (PQ2148-s6), which showed a much tighter repression in reporter gene experiments. While the tunability is preserved, the maximum gene expression strength is decreased compared to the original promoter. Therefore, the system is suitable for the expression of cytotoxic genes.

In summary, we could expand the genetic toolbox for *M. extorquens*, namely by a tightly regulatable promoter and a secondary plasmid system. This gives new possibilities for designing efficient production strains for methylotrophic biotechnology.

---

### 621-SMP

Dissemination of iron-scavenging pyoverdine as a model for public good-based interspecies communication within microbial communities

\*L. Kruse<sup>1</sup>, F. Hilgers<sup>1</sup>, O. Klaus<sup>1</sup>, V. Svensson<sup>1</sup>, K. E. Jaeger<sup>1,2</sup>, T. Drepper<sup>1</sup>

<sup>1</sup>Institute of Molecular Enzyme Technology, Heine-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

<sup>2</sup>Institute of Bio- and Geosciences IBG-1, Forschungszentrum Jülich, Jülich, Germany

Microbial communities are complex life forms that can be found in almost any habitat. Based on their metabolic versatility, these consortia play a superior role in essential biological processes like detoxification, food production or pathogenesis. In microbial communities, interspecies communication can be conveyed by the exchange of so-called public goods - beneficial compounds synthesized by an individual species that can subsequently be shared among the entire microbial consortium. Based on the properties of public goods, such social interactions can strongly impact dynamics and behaviour of the entire microbial community. In order to gain deeper insights into public good-based communication processes, the exchange of iron-scavenging compounds was initially analysed as a model system in a synthetic consortium of *Pseudomonas putida* and *Rhodobacter capsulatus*. Previous investigations have shown that *P. putida* represents an ideal model organism as public good producer, because it secretes the fluorescent siderophore pyoverdine under iron depletion. Released pyoverdine can efficiently bind Fe<sup>3+</sup> and subsequently be reimported. In contrast, bioinformatic analysis of the *R. capsulatus* genome sequence revealed the presence of three putative pyoverdine receptors genes, which might be involved in iron homeostasis<sup>1</sup>. Remarkably, no corresponding pyoverdine synthesis genes could be identified in the *R. capsulatus* genome making it an ideal public good-receiver candidate. To initially analyse the potential role of pyoverdine for iron homeostasis of *R. capsulatus*, cell growth of the wild-type strain SB1003 was analysed under increasing iron limitation in comparison to the *P. putida* wild-type KT2440 and the pyoverdine mutant ( $\Delta pvd$ ). As expected, iron deficiency resulted in a strong growth impairment of *R. capsulatus* and *P. putida*  $\Delta pvd$ . Remarkably, growth of *R. capsulatus* could be restored under these conditions by either adding cell-free pyoverdine-containing supernatant of *P. putida* wild-type cultures or in

respective co-cultivation experiments. These findings indicate that pyoverdine can act as a public good in this synthetic microbial consortium.

<sup>1</sup>Zappa *et al.* 2013

---

### 622-SMP

Towards the Reconstitution of Cryptophycin Biosynthesis

\*A. Tippelt<sup>1</sup>, M. Nett<sup>1</sup>

<sup>1</sup>TU Dortmund, Department of Biochemical and Chemical Engineering - Technical Biology, Dortmund, Germany

Cyanobacteria are versatile producers of structurally diverse secondary metabolites with clinical potential as antitumor agents (1). However, the genetic manipulation of these microorganisms is challenging. Likewise, cultivation on an industrially relevant scale is rarely feasible. The reconstitution of biosynthetic pathways to cyanobacterial natural products in more amenable hosts, such as *Saccharomyces cerevisiae*, is thus highly desirable.

In this project, we aim to generate a yeast strain for the production of cryptophycins, which are highly active, multidrug-resistant antitumor agents from the cyanobacterium *Nostoc* sp. ATCC 53789 (2). For this, we refactored the cryptophycin pathway. The four core biosynthetic genes *crpA-D* were equipped with artificial expression control elements (3,4) and, subsequently, inserted into the genome of a yeast strain expressing a broad spectrum 4'-phosphopantetheinyl transferase. The chromosomal integration was achieved by CRISPR-Cas9 and by homologous recombination into LTR sites (5,6). Subsequent genetic analyses confirmed the successful reconstitution and transcription of the cryptophycin genes in the recombinant yeast.

Currently, procedural improvements are carried out to increase the cryptophycin production titer. Furthermore, a directed precursor feed is implemented, exploiting the substrate tolerance of the biosynthetic enzymes. The objective is to generate non-natural cryptophycins with improved pharmaceutical properties.

(1) Kehr, J.-C. and Dittmann, E. *et al.* (2011) *Beilstein J. Org. Chem.* 7, 1622–1635.

(2) Magarvey, N. A. and Sherman, D. H. *et al.* (2006) *ACS Chem. Biol.* 1, 766–779.

(3) Redden, H. and Alper, H. S. *et al.* (2015) *Nat. Commun.* 6, 7810.

(4) Curran, K. A. and Alper, H. S. *et al.* (2015) *ACS Synth. Biol.* 4, 824–832.

(5) Reider Apel, and Mukhopadhyay, A. *et al.* (2017) *Nucleic Acids Res.* 45, 496–508.

(6) Bai Flagfeldt and Nielsen J. *et al.* (2009) *Yeast* 26, 545–551.

---

### 623-SMP

Redesigning hydrogenase: a semisynthetic [Mn]-hydrogenase

\*G. Huang<sup>1</sup>, H. Pan<sup>2</sup>, M. D. Wodrich<sup>2</sup>, F. F. Tirani<sup>2</sup>, K. Ataka<sup>3</sup>, X. Hu<sup>2</sup>, S. Shima<sup>1</sup>

Hydrogenases, a group of metalloenzymes, catalyze H<sub>2</sub> production and utilization. In nature, only Ni and/or Fe are employed as the active-site metals for [NiFe]-, [FeFe]- and [Fe]-hydrogenase. However, many synthetic transition-metal mimic-compounds are able to catalyze H<sub>2</sub>-activation. [Fe]-hydrogenase, mainly found in methanogenic archaea, reversibly catalyzes the hydride transfer from H<sub>2</sub> to an organic hydride acceptor, methenyl-tetrahydromethanopterin. The prosthetic group of [Fe]-hydrogenase is the iron-guananylylpyridinol (FeGP) cofactor, which contains mono-iron center playing a key role in H<sub>2</sub> activation [1,2]. Here, we report a semisynthetic [Mn]-hydrogenase, which is made of a redesigned model compound of the FeGP cofactor, in which Fe(II) was replaced by Mn(II) [3]. This semisynthetic [Mn]-hydrogenase performed heterolytic cleavage of H<sub>2</sub> and hydrogenation as native [Fe]-hydrogenase. Notably, this non-native [Mn]-hydrogenase showed higher catalytic activity than the semi-synthetic [Fe]-hydrogenase in the occupancy-normalized case [3,4]. This finding provides information that synthesis of a non-native metal hydrogenase with H<sub>2</sub>-activation/generation functions is possible. Additionally, it opens a general question: why nature does not consider Mn as hydrogenase metal site?

[1] Shima S, Pilak O, et al. *Science* 2008, 321(5888): 572-575.

[2] Huang G, Wagner T, et al. *Nat. Catal.* 2019, 2(6): 537-543.

[3] Pan HJ, Huang G, et al. *Nat. Chem.* 2019, 11(7): 669-675.

[4] Shima S, Chen D, et al. *Nat. Chem.* 2015, 7(12): 995-1002.

## 624-SMP

### ***Rhodobacter capsulatus* as a phototrophic platform organism for the synthesis of plant terpenes**

\*O. Klaus<sup>1</sup>, A. Loeschcke<sup>1</sup>, F. Hilgers<sup>1</sup>, J. Hage-Hülsmann<sup>2</sup>, K. Troost<sup>2</sup>, K. E. Jaeger<sup>1,3</sup>, T. Drepper<sup>1</sup>

<sup>1</sup>Institute of Molecular Enzyme Technology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

<sup>2</sup>Institute of Molecular Enzyme Technology, Heinrich-Heine-University Düsseldorf, Forschungszentrum Jülich, Jülich, Germany

<sup>3</sup>Institute of Bio- and Geosciences IBG-1, Forschungszentrum Jülich, Jülich, Germany

Terpenes are a large class of natural compounds that can be used, for example, as fragrances, cosmetics or pharmaceuticals in different industrial and biomedical applications. Due to its phototrophic lifestyle, *Rhodobacter capsulatus* is a versatile host that can be used for the production of plant-derived terpenes. The formation of an intracytoplasmic membrane system as well as an efficient intrinsic tetraterpene pathway are unique properties of this strain that are favorable for the synthesis and storage of these hydrophobic natural products. In addition, *R. capsulatus* can form polyhydroxybutyrate (PHB) granules under phototrophic conditions. These microbial intracellular inclusions gained biotechnological interest because they can be used as a matrix for *in vivo* immobilization of heterologous proteins.

Here, we present different strategies to engineer *R. capsulatus* as a phototrophic platform organism for the synthesis of sesqui-, di-, tri- and tetraterpenes. To analyze if gradually adjustable formation of the key precursor farnesylpyrophosphate (FPP) is beneficial for terpene synthesis under phototrophic conditions, the genes of the intrinsic 1-deoxy-d-xylulose 5-phosphate (DXP) pathway as well as the heterologous mevalonate (MVA) pathway were modularly co-expressed with the respective terpene synthase gene in various combinations. Notably, these comparative studies identified a distinct combination of precursor biosynthetic genes as best-performing setup for most of the tested terpene synthases. To analyze the effect of enzyme co-localization on terpenoid production we finally generated expression plasmids allowing the *in vivo* immobilization of proteins on the PHB surface in *R. capsulatus*. As proof of concept, we used the PHB synthase PhaC as an appropriated PHB anchor protein and the yellow fluorescent protein EYFP as target protein. The modular engineering approaches together with the PHB-mediated control of spatial arrangement developed in this study can further help to establish *R. capsulatus* as a phototrophic platform organism for the production and characterization of new plant-derived terpenes.

## 625-SMP

Whole-cell biosensors as effective, fast and low-cost tool in antibiotic research

D. Wolf<sup>1</sup>, \*L. Friebe<sup>1</sup>, D. Iliasov<sup>1</sup>, A. Walder<sup>1</sup>

<sup>1</sup>Technische Universität Dresden, Chair of General Microbiology, Dresden, Germany

Considering the increase of multi-resistant bacterial pathogens to available antimicrobials, the necessity for novel antibiotic substances is becoming urgent. Genome mining, synthetic chemistry and re-focusing efforts to new groups of producing organisms has provided many new sources for antibiotics that need to be efficiently characterized for their mode of action. Whole-cell biosensors can be used as an effective tool to screen novel antimicrobial substances for their cellular target. Biosensors are microbial reporter strains that combine an antibiotic-responsive input module (the sensor) with an automatically quantifiable output module (the reporter) [1]. In our lab, we have developed a range of *Escherichia coli* and *Bacillus subtilis* biosensors that cover all major classes of antimicrobial targets in bacteria. Here, we present a selection of newly developed biosensors, which responds to antibiotics in a high and specific manner. The biosensor constructs generated each consists of the promoter of a gene that, based on gene expression analysis and transcriptome studies, showed an increased expression under antibiotic stress. The strength of the biosensors' promoter was tested through induction by adding appropriate antibiotics, followed by measurement of luminescence using plate reader assays. We found that the promoter P<sub>recA</sub>, P<sub>helD</sub>, P<sub>yrzI</sub> and P<sub>yuaF</sub> from *B. subtilis* specifically respond to norfloxacin, rifampicin, erythromycin and penicillin, respectively. Furthermore, we successfully established *E. coli* biosensors containing P<sub>uvrA</sub> or P<sub>tet</sub> as sensory unit for fluoroquinolones and tetracycline, respectively. Additionally, we also characterized these biosensors in more detail regarding their dynamics, specificity and strength. In summary, the presented biosensors enlarge our already existing panel of diverse biosensors to narrow down the cellular target of novel antibiotics in a fast and efficient way.

[1] Wolf & Mascher (2016) *Appl Microbiol Biotechnol* 100:4817-29

## 626-SMP

Converting a non-magnetotactic species of *Magnetospirillum* into a magnetosome-producer through a single-step transfer of biosynthetic magnetosome operons

\*M. Dziuba<sup>1,2</sup>, D. Schüler<sup>1</sup>

<sup>1</sup>University of Bayreuth, Microbiology, Bayreuth, Germany

<sup>2</sup>Research Center of Biotechnology RAS, Institute of Bioengineering, Moscow, Russian Federation

Magnetotactic bacteria (MTB) are known for their unique ability to synthesize complex membrane-enveloped magnetic organelles, magnetosomes, which they use for magnetic navigation. The best studied MTB belong to genus *Magnetospirillum*, which besides numerous magnetotactic strains also contains an increasing number of non-magnetotactic species lacking genes for magnetosome formation. Both types are abundant in aquatic sediments. Their close phylogenetic relationship raised the questions: (i) did the non-magnetotactic magnetospirilla lose magnetosome genes during evolution? (ii) Can they be (re-)magnetized by transfer of magnetosome genes? Previously, this could be demonstrated only for a single species (*Rhodospirillum rubrum*), while failed in other tested *Alphaproteobacteria*. In this study we assessed the potential of non-magnetotactic *Magnetospirillum* spp. to synthesize magnetosomes upon acquisition of the corresponding genes from a related species. A compact vector harbouring the set of magnetosome operons (>30 genes) from *Magnetospirillum gryphiswaldense* MSR-1 was transferred in a single-step into three non-magnetotactic strains *Magnetospirillum* sp. 15-1, *M. aberrantis* SpK and *M. bellicus* VDY. While SpK and VDY could not be transformed due to the presence of a putative restriction-modification system(s), conjugative transfer of the vector induced magnetosome biosynthesis in the hitherto non-magnetotactic strain *Magnetospirillum* sp. 15-1. However, the magnetosomes produced by the obtained mutants were smaller and poorly organized within the cells that resulted in the lack of efficient magnetotaxis in weak magnetic fields (of  $\mu$ T range). This might be attributed to the lower expression levels of several magnetosome proteins in strain 15-1. In summary, our findings showed that at least one of the non-magnetotactic species of *Magnetospirillum* can be "magnetized" by the transfer of magnetosome genes from a magnetotactic strain MSR-1. We also demonstrate potential limitations in the natural ability of horizontally transferred magnetosome genes to execute their function in a new host, even if it is closely related.

## 627-SMP

Biosynthesis of sphingofungins in human pathogenic fungus *Aspergillus fumigatus*

\*A. U. Bissell<sup>1</sup>, J. Rautschek<sup>1</sup>, J. E. Kufs<sup>1</sup>, D. J. Mattern<sup>1</sup>, S. Hoefgen<sup>1</sup>, Y. Huang<sup>1</sup>, A. A. Brakhage<sup>1</sup>, V. Valiante<sup>1</sup>

<sup>1</sup>Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie, Hans-Knöll-Institut, Biobricks mikrobieller Naturstoffsynthesen, Jena, Germany

Secondary metabolites produced by filamentous fungi are a vast and still not fully explored reservoir of biologically active molecules. While many obvious candidates have been extensively studied there are still many compounds left of which there is little known. Among these compounds is a class of molecules categorized as sphingolipid inhibitors (SIs). These inhibitors are considered promising candidates for new therapeutic approaches to intervene in the sphingolipid homeostasis, which is imbalanced in numerous human diseases such as diabetes and Alzheimer's disease.

While the biosynthesis of the SI fumonisin has been thoroughly studied in *Fusarium*, the overall knowledge on other SIs is still limited. To rectify this gap of knowledge we

have begun to investigate the sphingofungins produced by *Aspergillus fumigatus*, which are SIs structurally related to fumonisins. Certain similarities of gene sequences and gene cluster composition to the fumonisin biosynthetic genes have led us to identify the biosynthetic gene cluster of sphingofungins, as well as to establish an overexpression mutant of the cluster for higher sphingofungin production. In combination with deletion mutants of all genes assigned to the biosynthetic cluster we are well on our way to characterize the biosynthesis of sphingofungins and its intermediates in *A. fumigatus*.

The analysis of genome databases has suggested that there are more than three hundred putative SI producing gene clusters in fungi. So we want to use our knowledge gained on the general aspects of SI biosynthesis to produce novel compounds by combining biosynthetic SI producing genes of different fungal organisms.

## 628-SMP

Targeted metabolite recovery from synthetic microbial communities resembling anaerobic digesters

\*C. Möslinger<sup>1</sup>, J. Dolinsek<sup>1</sup>

<sup>1</sup>Kompetenzzentrum Holz, Holzchemie und Biotechnologie, Linz, Austria

### Introduction

Biorefineries aim to convert lignocellulosic biomass, the world's most abundant biobased renewable resource, into valuable bulk chemicals. Microbes grow at mild conditions, thus biotechnology holds a promise to make biorefinery processes cheaper and more environmentally friendly. Anaerobic digesters are mature technology where diverse microbes convert lignocellulosic biomass into methane gas. Besides methane, a number of valuable compounds such as lactic acid are continuously produced in anaerobic digesters, but are also rapidly metabolized by microbes such as acetogens. While such rapid turnover prevents toxic metabolites to build up, it also prevents recovery of valuable chemicals.

### Objectives

The aim of this work is to rationally create a synthetic microbial community that resembles communities in anaerobic digesters but is capable to efficiently convert lignocellulosic biomass into valuable compounds other than methane gas.

### Materials & Methods

*Spirochaeta thermophila* can convert lignocellulose into D-lactate, H<sub>2</sub>, and acetate. D-lactate and acetate inhibit growth - their removal should improve further substrate turnover. *Methanotrix thermoacetophila* is an acetoclastic methanogen that converts acetate into CH<sub>4</sub>. *M. thermoacetophila* neither metabolizes *S. thermophila*'s substrates, nor does it metabolize the desired products H<sub>2</sub> and D-lactate. We expect that when combined, both microbes will convert lignocellulose into products D-lactate, H<sub>2</sub>, and CH<sub>4</sub>.

### Results

*S. thermophila* rapidly acidifies its environment, and product inhibition prevents further glucose turnover. Models suggest that the interaction between both microbes becomes increasingly mutualistic as pH decreases and metabolites

lactate and acetate become increasingly toxic. *M. thermoacetophila* removes acetate and thus allows *S. thermophila* to grow at low pH where *S. thermophila* cannot grow on its own.

## Conclusion

We hope to build a community that converts lignocellulose into valuable products D-lactate, CH<sub>4</sub> and H<sub>2</sub>. Such process could replace oil-based processes and contribute to CO<sub>2</sub> reduction.

---

## 629-SMP

### ***small noncoding RNAs as a tool to modulate gene expression***

\*P. Apura<sup>1</sup>, A. Peregrina<sup>1</sup>, M. Saramago<sup>1</sup>, S. C. Viegas<sup>1</sup>, S. M. Carvalho<sup>1</sup>, L. M. Saraiva<sup>1</sup>, S. M. Domingues<sup>1</sup>, C. Arraiano<sup>1</sup>  
<sup>1</sup>ITQB NOVA, Oeiras, Portugal

Bacteria have been used to produce all kinds of compounds with industrial interest. However, their efficiency is compromised by their complex metabolism and low survival in the demanding industrial conditions.

Small noncoding RNAs (sRNAs) constitute functional RNA molecules, that are involved in cellular functions that go from ribosomal modification, participation in catalytic biochemical reactions to regulation at the transcriptional, post-transcriptional, translational and post-translational levels. These molecules appear to be promising regulatory agents which can be transferred between different bacterial species and designed to target expression of specific genes. Modulation of gene expression is an urgent need to redesign metabolism of industrial microorganisms.

Here we report the construction of a small RNA-based regulatory system with potential to be used for different purposes in synthetic biology. Our model microorganism is *Pseudomonas putida*, a very stress-resistant bacterium and a good candidate to be exploited in the harsh conditions of industrial bioreactors. The customized sRNAs here developed were implemented as a tool to modulate the expression of endogenous (*acnB* and *sdhB*) and reporter genes (*gfp* and *yfp*). These customized sRNAs are composed by two parts: i) constant part composed by a scaffold structure that is responsible for the recruitment of the Hfq, facilitating the binding of the sRNA to the target; ii) flexible part composed by a sequence that is complementary to the chosen target mRNA. The novelty of our work resides in the duality of this plasmid expression system that not only can downregulate a chosen gene but also overexpress it. This can be accomplished by simply changing the region of the antisense interaction of the sRNA with the target mRNA transcript.

By plugging-in and -out genetic circuits, this tailor-made regulatory system can be used to redesign *P. putida* metabolism, fulfilling an important industrial gap.

---

## 630-SMP

The author has not agreed to a publication.

---

## 631-SMP

Tailored engineering of fungal nonribosomal peptide synthetases to obtain artificial cyclodepsipeptides

\*C. Steiniger<sup>1,2</sup>, S. Hoffmann<sup>1</sup>, A. Mainz<sup>1</sup>, M. Kaiser<sup>3</sup>, K. Voigt<sup>4</sup>, V. Meyer<sup>2</sup>, R. D. Süssmuth<sup>1</sup>

<sup>1</sup>Berlin Institute of Technology, Dep. Biological Chemistry, Berlin, Germany

<sup>2</sup>Berlin Institute of Technology, Dep. Applied and Molecular Microbiology, Berlin, Germany

<sup>3</sup>Swiss Tropical and Public Health Institute, Basel, Switzerland

<sup>4</sup>Leibniz-Institute for Natural Product Research and Infection Biology, Jena, Germany

Nonribosomal peptide synthetases (NRPSs) represent promising engineering platforms for the design of structurally complex peptides of pharmacological importance. While previous studies focused on bacterial synthetases, fungal systems assembling drugs like the antibacterial cephalosporins, antifungal echinocandins or immunosuppressive cyclosporine still await in-depth exploitation. In the forefront of our studies, an effective engineering of fungal iterative cyclodepsipeptide (CDP) synthetases in particular has been severely hampered, as various mechanistic features of CDP biosynthesis were only poorly understood.

By combining protein truncation, *in trans* expression and combinatorial biosynthesis, we assigned important functional segments of fungal CDP synthetases. The gained mechanistic knowledge allowed for the design of a variety of *in vivo* functional artificial assembly lines comprising parts from up to three different NRPSs. By active CDP ring size control and the application of the Tet-on expression system in *Aspergillus niger* as a heterologous host, we obtained the new-to-nature CDPs octa-enniatin and octa-beauvericin, as well as high titers of hexa-bassianolide (g/L scale), which showed superior antiparasitic activity accompanied by cytotoxicity.

As an alternative NRPS engineering approach, we fused up to three CDP synthetases by harnessing their unique terminal C domain as canonical C domain. This induced a switch from an exclusively iterative to a mixed iterative/linear assembly mode, leading to various hybrid CDPs with altered symmetry. Based on a systematic comparison of three fungal NRPS exchange units (C-A-Mt-T, CCTD-A-Mt-T, A-Mt-T), we also demonstrate that fungal NRPSs of different assembly types can be combined using different swapping sites, while respecting the C domain integrity and specificity. Overall, our findings give distinct clues for efficient reprogramming of iterative and linear fungal NRPSs in future protein engineering approaches.

---

## 632-SMP

### ***Rapid purification and characterization of recombinant proteins and antibodies: Capturem high-capacity membranes***

G. Jokhadze<sup>1</sup>, C. Hopmann<sup>1</sup>, M. T. Vierra<sup>1</sup>, B. Levitan<sup>1</sup>, M. Li<sup>1</sup>, A. Farmer<sup>1</sup>, \*M. Raman<sup>2</sup>

<sup>1</sup>Takara Bio USA, Mountain View, United States

<sup>2</sup>Takara Bio Europe SAS, Saint-Germain-en-Laye

Recombinant protein production is immensely important in many research settings, including academic research institutions, biopharmaceutical organizations, and enzyme and agricultural industries. Fusion tags are widely used to improve yields and enable purification and characterization of protein structure and function. Polyhistidine tags, which incorporate 6–10 histidines at either terminus of the target proteins, are the most popular tag used for purification. The affinity of the histidines to immobilized metal ions such as Co<sup>2+</sup> and Ni<sup>2+</sup> is utilized to selectively bind the tagged construct to the matrix, while washing away unwanted materials, before eluting the target with low-pH or imidazole-

containing buffers. Typical purification methods using immobilized metal affinity chromatography (IMAC) columns take several hours to complete due to long column equilibration/binding times and slow diffusion of large macromolecules through the resin bed. The long times increase the risk of proteolytic degradation and activity loss due to unfolding or denaturation. Membrane-based affinity systems have rapid flow-induced mass transport and short residence times; however, they have been plagued with low capacity due to small internal surface areas. Here, we describe a novel, nylon-membrane-based IMAC system with chemically enhanced surface areas of the pores that allow protein binding capacities comparable to, or better than, resins at 75 mg or more per cm<sup>3</sup> of membrane. Unlike traditional resin-based systems, the entire purification process—from loading the lysate to eluting pure protein—can be completed at room temperature in less than five minutes. We have assembled these membranes into spin columns and filtration devices and demonstrated their ability to purify histagged proteins produced in bacterial and mammalian cells. The millisecond residence time of the proteins on the membrane during binding minimizes the possibility of degradation. These membranes function perfectly in the presence of additives such as ethylenediaminetetraacetic acid (EDTA), reducing agents such as dithiothreitol (DTT), and under denaturing conditions (in the presence of urea and guanidium hydrochloride). We have extended the high-capacity membrane technology to immobilize Protein A and G, enabling extremely fast purification of antibodies from various matrices based on the affinity of these proteins for the fragment crystallizable region (Fc) region of antibodies. Antibody purification can be accomplished in less than 15 minutes, with capacities of up to 40 mg/ml or more, far exceeding the capacity of resin-based columns. More recently, we have immobilized trypsin and pepsin enzymes on these membranes to carry out proteolysis of proteins for their characterization, identification, and quantitation through mass spectrometry analysis. In contrast to the long incubation periods (6–24 hours) of conventional in-solution digestions, the proteolytic membranes generate peptides suitable for downstream analysis, with equivalent or improved sequence coverage, in less than a minute. Additionally, we expanded the membrane technology with immobilized streptavidin suitable for enrichment of target proteins, antibodies, and oligos. These novel membrane-based spinable affinity columns and filtration devices will be useful for purifying a variety of recombinant proteins and antibodies and their proteomics characterization in academic and industrial settings.

### 633-MEEP

Unicellular Labyrinthulomycetes Protists are a key Component of Heterotrophic Microbes in the Coastal Oceans  
N. Xie<sup>1</sup>, M. Bai<sup>1</sup>, L. Liu<sup>1</sup>, \*G. Wang<sup>1</sup>

<sup>1</sup>Tianjin University, Environmental Science & Ecology, Tianjin, China

Heterotrophic microbes play a key role in remineralizing organic matters and secondary production in the coastal oceans. While there is a significant body of literature examining of bacterial and phytoplankton communities, much less is known about the diversity, dynamics and ecology of eukaryotic heterotrophs. Labyrinthulomycetes are unicellular fungus-like protists with the ubiquitous presence in the oceans, but whose ecology is poorly understood. Our recent studies indicate that the biomass of this group of protistan microbes can exceed that of the bacterioplankton in coastal waters. Like bacterioplankton, their community is dominated by a few prevalent taxa with summer or winter preferences. However, the majority of phylotypes occurs mostly as reoccurring, season-specific blooms as previously described for planktonic fungi. Clearly, niche partitioning in responding

to environmental variables commonly occurs among closely-related Labyrinthulomycetes protists. Their distinct temporal and spatial distributions in the coastal waters compared to bacterioplankton and planktonic fungi suggest the dynamic relationship among these heterotrophic microbial groups and their significant contributions to the total rates of carbon and nutrient cycling in the coastal oceans.

### 634-MEEP

Fast and universal metaproteome workflow for analysis of microbial communities

R. Heyer<sup>1</sup>, K. Schallert<sup>1</sup>, A. Büdel<sup>1</sup>, R. Zoun<sup>2</sup>, S. Dorl<sup>3</sup>, A. Behne<sup>4</sup>, F. Kohrs<sup>1</sup>, S. Püttker<sup>1</sup>, C. Siewert<sup>5</sup>, T. Muth<sup>6</sup>, G. Saake<sup>2</sup>, U. Reichl<sup>1,5</sup>, \*D. Benndorf<sup>1,5</sup>

<sup>1</sup>Otto von Guericke University, Bioprocess Engineering, Magdeburg, Germany

<sup>2</sup>Otto von Guericke University, Institute for Technical and Business Information Systems, Magdeburg, Germany

<sup>3</sup>University of Applied Sciences Upper Austria, Bioinformatics Research Group, Hagenberg, Austria

<sup>4</sup>glyXera GmbH, Magdeburg, Germany

<sup>5</sup>Max Planck Institute for Dynamics of Complex Technical Systems, Bioprocess Engineering, Magdeburg, Germany

<sup>6</sup>Robert Koch Institute, Department for Methods Development and Research Infrastructure, Bioinformatics Unit, Berlin, Germany

### Question

Metaproteomics provides valuable insight into structure and function of microbial communities in the human body, in natural habitats, and in technical systems. Its application as a diagnostic tool in medicine and biotechnology, however, is still hampered by labor and time-consuming experimental workflows and by extremely high computational efforts for bioinformatic data analysis. To tackle these issues, a metaproteomics workflow was established that includes a high-throughput and standardized sample preparation, up-to-date mass spectrometry and fast bioinformatic algorithms, and overcomes limitations of previous solutions.

### Methods

A combination of phenol extraction and FASP digestion of extracted proteins allowed the preparation of samples from human faeces, soils, wastewater treatment plants and biogas plants in less than 13 h for a single sample, limited only by the LC-MS/MS run time. We developed a new version of the MetaProteomeAnalyzer for qualitative and quantitative analysis of taxonomic and functional data. The software is provided to the scientific community as a central remote server solution ([www.mpa.ovgu.de](http://www.mpa.ovgu.de)).

### Results

The improved workflow generated at least two times as many protein identifications than the previous workflow, and a drastic increase of taxonomic and functional annotations. Technical replicates of samples showed high reproducibility (more than 90% identical metaproteins, Pearson coefficients higher than 0.9), whereas different types of samples were clearly separated (less than 70% identical metaproteins, Pearson coefficients lower than 0.7).

### Conclusion

Improved sample preparation and bioinformatic analysis provided taxonomic and functional data of microbial communities in less than 24 hours demonstrating the potential to compete with metagenomic or



metatranscriptomic approaches when applied as diagnostic tool in medicine or biotechnology.

---

### 635-MEEP

Altered nutrient levels and ratios in freshwater microcosms result in context dependent responses of microbial communities

\*A. Kuppardt-Kirmse<sup>1</sup>, C. Karakoç<sup>1,2</sup>, A. Chatzinotas<sup>1,2</sup>

<sup>1</sup>Helmholtz Centre of Environmental Research GmbH - UFZ, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>German Centre for Integrative Biodiversity Research (iDiv), Leipzig, Germany

Changes in nutrient levels and ratios are recognized as one of the major components of global change in freshwater ecosystems. Revealing the impact of nutrient load and altered elemental ratios in the nutrient supply on freshwater bacterial communities is of paramount importance to further our understanding of contemporary and future freshwater ecosystems. Using microcosm experiments established with freshwater microbial communities we investigated the impacts of elevated nitrogen concentration on microbial diversity and community composition on the DNA (i.e. total community) and the RNA level (i.e. potentially active community) by sequencing and T-RFLP fingerprinting. In addition we assessed bacterial community function with community level physiological profiling. In order to infer the impact of nutrient ratio, we applied nitrogen elevation with or without a phosphorus supply. We found that different community measures showed distinct responses. While bacterial diversity was only affected by nutrient ratio, community composition and function were both sensitive to nutrient ratio and levels of nutrient elevation, as well as their interactive effects. Correlation between function and community composition were dependent on the nutrient ratios and the community of interest (i.e. potentially active or total community). Overall, our study stresses the importance of further considering the impact of nutrient imbalances on microbial communities, since they might cause serious alterations of freshwater ecosystem functioning under global change.

---

### 636-MEEP

**Succession or co-blooming in plankton – Correlating genetic distance and fitness difference**

\*J. Hoffmann<sup>1</sup>, K. Vergin<sup>2</sup>, S. Giovannoni<sup>2</sup>, F. Hellweger<sup>1</sup>

<sup>1</sup>Technische Universität Berlin, Wasserreinhaltung, Berlin, Germany

<sup>2</sup>Oregon State University, Corvallis, Germany

Driven by advances in technology and need (e.g. identifying toxigenic and non-toxigenic genotypes) microbial observations are evolving to become increasingly resolved (micro-diversity). These high-resolution observations (e.g. genotypes) are challenging the traditional model of phytoplankton species succession because they frequently show great sequence diversity indicating potential co-blooming.

We hypothesize that co-blooming types (species, strains, genotypes, etc.), occupying similar ecological niches, are evolutionarily more related, and thus genetically closer than those showing succession. We test this hypothesis using a meta-analysis of plankton datasets. Specifically, we analyze marine and freshwater datasets and correlate fitness difference to genetic distance.

To quantify fitness differences, a selection coefficient (average population growth rate similarity) is calculated for

all pairs of types within each dataset. Our measure can be applied to absolute and relative abundance data.

Genetic distance is quantified using 16S rRNA average nucleotide distances (16S ANDs). For closely related types we base the distance on the 16S rRNA internally transcribed spacer (ITS) region and convert it to 16S AND. Conversion factors were obtained by creating libraries of species that allowed for calculation of 16S AND as well as 16S ITS AND. To allow for comparison of eukaryotes and prokaryotes, rubisco subunit RbcL protein sequences or plastid 16S rRNA gene sequences were used and similarly converted.

Application of the method to several marine and freshwater datasets revealed a weak but consistently positive correlation between genetic distance and fitness differences. On a larger scale, these results could contribute to improved models that may address basic principles of diversity, ecology and evolution.

---

### 637-MEEP

Different microbial community structures of a late decaying community change interaction patterns during decomposition of *Fagus sylvatica* under two temperature regimes

\*S. Muszynski<sup>1</sup>, F. Maurer<sup>1</sup>, M. Noll<sup>1</sup>

<sup>1</sup>Coburg University of Applied Sciences, Institute for Bioanalysis, Coburg, Germany

Ecosystems processes are driven by fluctuating temperature regimes, which affects ecosystem functions and biodiversity. To understand underlying ecological processes the insurance hypothesis was postulated, which state a higher resilience and species richness in fluctuating temperature regimes. In this study, the effect of community composition to cope such regimes during natural wood decay was analysed. Therefore, a late decaying indigenous (i) and diversity-reduced (d) microbial community composition of beech (*Fagus sylvatica*) were incubated for eight weeks under a constant (= 10°C) and a fluctuating (Ø 10°C) temperature regime. Microbial activity (wood mass loss and wood pH) together with bacterial and fungal biodiversity and its interaction pattern (network analysis) were assessed. Results revealed significant differences in microbial activity between (i) and (d) community compositions, but temperature regime had no effect on microbial productivity in terms of wood decomposition. Fungal community composition was strongly associated to (d), but not to (i) community composition, while the bacterial community is neither affected by (d) or (i). First facile insights in interaction pattern provided bacterial and fungal shifts over eight weeks for (i) and (d) community composition. As result the bacterial and to a lesser extend fungal community composition was highly resilient and functional fungal key-players were promoted by a fluctuating temperature regime. Such key-players were less integrated in microbial networks indicating a benefit for few fungi in this ecological niche, which was used for an opportunistic lifestyle.

---

### 638-MEEP

Monitoring seasonal changes in diversity, community structure and sedimentary deposition of cyanobacteria in Lake Tiefer See, NE Germany.

\*E. Nwosu<sup>1</sup>, A. Brauer<sup>2,3</sup>, D. Wagner<sup>2,3</sup>, S. Yang<sup>2</sup>, E. Dittmann-Thünemann<sup>4</sup>, S. Liebner<sup>2,5</sup>

<sup>1</sup>Helmholtz Zentrum, Deutsches Geoforschungszentrum GFZ Potsdam, Geomikrobiologie, Potsdam, Germany

<sup>2</sup>German Center for Geosciences, Potsdam, Geomicrobiology, Potsdam, Germany

<sup>3</sup>University of Potsdam, Institute of Geosciences, Potsdam, Germany

Limnic cyanobacterial populations can be signatures of past and recent environmental change and have been reconstructed from lake sediments since the begin of industrialization. What remains unknown is in how far sedimentary cyanobacterial DNA of centennial reconstructions resembles those of the water column, and what percentage of water column communities is eventually preserved in the sediments given that most cyanobacteria do not produce durable remains that preserve in the sediments.

In this study, we surveyed the seasonal dynamics of cyanobacterial communities via 16S rRNA gene Illumina sequencing in different water column depths of the meta- and hypolimnion and in surface sediments in order to track the dominant cyanobacterial groups in Lake Tiefer See and their sedimentary deposition. Furthermore, we present data showing how present-day water column cyanobacterial communities correlate to those from sedimentary reconstruction over the past 350 years.

Microscopic observations from the bloom period (early August 2019) revealed peaks of filamentous potential microcystin producing taxa assigned to *Anabaena* and *Dolichospermum*. Other filamentous cyanobacteria like *Phormidium* and picoplankton cyanobacteria were also observed, however, in low abundance. This cyanobacterial community observation in the water column were generally similar to those reconstructed from a sediment core spanning the last 350 years from Lake Tiefer See, where amplicon single variants (ASVs) assigned to unicellular picoplankton *Cyanobium* and *Aphanizomenon* belonging to the ADA clade (*Anabaena*, *Dolichospermum*, and *Aphanizomenon*) were equally the most dominant picoplankton and filamentous cyanobacteria, respectively. Further analyses of the 16S rRNA gene cyanobacterial community of the water column are still in progress.

Our study is the first to quantify seasonal and spatial changes of cyanobacteria in Lake Tiefer See and contributes to a better understanding of current and past environmental changes of this lake.

## 639-MEEP

Comparative genomics reveal insights into genomic plasticity and habitat adaptation of ***Streptococcus parasanguinis***

\*M. M. H. Abdelbary<sup>1</sup>, G. Wilms<sup>1</sup>, G. Conrads<sup>1</sup>

<sup>1</sup>RWTH Aachen University Hospital, Division of Oral Microbiology and Immunology, Department of Operative and Preventive Dentistry and Periodontology, Aachen, Germany

**Introduction:** *Streptococcus parasanguinis* is a commensal that colonizes the oral cavity, but also commonly found in the gastrointestinal tract (stomach and small intestine) of healthy adults. However, the mechanisms underlying within-host adaptation of *S. parasanguinis* to different habitats remain unstudied.

**Objectives:** We aimed to elucidate the population structure of *S. parasanguinis* and to determine the evolutionary pathways that allow strains of oral origin to adapt to the gut environment.

**Materials & methods:** We performed a comparative genomic analysis on 44 *S. parasanguinis* genomes that were collected from various body sites and 8 different countries. Most of the genomes (n = 42) were retrieved from public

database, while the remaining two genomes were in-house collected from two habitats of the same patient and sequenced.

**Results:** We detected 14,446 single-nucleotide polymorphisms (SNPs) and a high level of recombination events among the core genomes of *S. parasanguinis*. Intestinal *S. parasanguinis* genomes harboured intact prophages that were phylogenetically distinct from their oral counterparts. Furthermore, a large genomic element (63 genes) that encodes for the anaerobic cobalamin/vitamin B12 synthesis was detected mainly in *S. parasanguinis* genomes of intestinal and clinical origin suggesting adaptation to the anaerobic condition including the gastrointestinal tract. Another genomic fragment composes of 16 genes that encode for an accessory secretion system (aSEC) found in nine *S. parasanguinis* genomes forming a distinct phylogenetic clade.

**Conclusion:** Our results revealed a high level of recombination among *S. parasanguinis* genomes suggesting frequent horizontal gene transfer. Mobile genetic elements have played an important role in the adaptation of *S. parasanguinis* to the intestinal habitat. Taken together, our findings emphasize the high degree of genomic plasticity of *S. parasanguinis* that might reflect special evolutionary processes enabling for adaptation to different ecosystems.

---

## 640-MEEP

The recovery of metagenome-assembled genomes is influenced by sample evenness.

\*J. P. Saraiva<sup>1</sup>, R. Brizola Toscan<sup>1</sup>, U. Nunes da Rocha<sup>1</sup>

<sup>1</sup>Helmholtz Center for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany

How do community diversity (e.g., species abundance, taxonomic relatedness) and sequencing depth impact the recovery of metagenome assembled genomes (MAGs) from complex microbial communities?

To answer this question we selected 12 phyla (9 Bacterial, 2 Archaeal and 1 Fungal) three times (three sets of richness). For each phylum, we selected 2 organisms of the same genus and one from a different family. Also, we introduced special genomic configuration (e.g., multiple and linear chromosomes). Forty two species were selected for each richness (126 in total), from which all genomes have been sequenced. Next, we simulated 23 metagenomes *in silico* varying evenness profiles and number of HiSeq Illumina reads for each richness set (69 in total) using MetaSim. We selected three supervised pipelines (two automated and one semi-automated) to recover MAGS from all simulated metagenomes (completeness and contamination were evaluated by CheckM).

We recovered over 5500 MAGs from the 69 *in silico* communities using the three recovery pipelines per *in silico* metagenome. Communities composed of taxonomically distinct species showed a statistically significant ( $p < 0.05$ ) increase in the number of recovered bins when compared to communities composed of taxonomically related species. A statistically significant increase ( $p < 0.05$ ) in the number of MAGs was only obtained when comparing sequencing depths of 10 versus 120 and 10 versus 180 million reads. Communities composed of taxonomically distinct species also showed a statistically significant increase ( $p < 0.05$ ) in genome completeness when compared to communities composed of taxonomically related microbes.

This study shows that the highest number of recovered genomes occurs when species present different relative abundances and they are not taxonomically related. This is because current tools are unable to distinguish between species with similar taxonomies (genus level) at comparable abundance levels. Further, increasing the sequencing depth over 60 million reads does not significantly improve genome recovery for the tested evenness distributions.

---

#### 641-MEEP

Coupling cell sorting and metagenomics allows the recovery of genomes from low abundance species

\*N. Abdulkadir<sup>1</sup>, J. Pedro Saraiva<sup>1</sup>, F. Schattenberg<sup>1</sup>, S. Müller<sup>1</sup>, U. Nunes da Rocha<sup>1</sup>

<sup>1</sup>Helmholtz Center for Environmental Research-UFZ, Environmental Microbiology, Leipzig, Germany

Flow cytometry has advantages over sequencing techniques by providing quantitative information of cell numbers which can further be used to define sub-communities. However, this technique does not provide information on function and phylogeny is usually assessed by 16S rRNA sequencing. Metagenomics is a standard technique used to study microbial community's composition and functional potential. The complexity and high diversity found in most ecosystems allow only the study of the dominant species through metagenomics. For example, in highly diverse environments only a few dozen species can be recovered as metagenome assembled genomes (MAGs) per sample.

We hypothesize it is possible to recover genomes from less abundant microbial species by recovering MAGs from different sub-community separated via flow cytometry. To test this hypothesis, we sorted a wastewater microbial community in dominant (DP), rare (PR) and outer gates (O) while the unsorted (U) community was used as a control. Next, DNA extraction and sequencing was performed on cells from the different sub-communities and the control. Metagenomes were sequenced (150 bp paired-end Illumina reads, minimum 20 million reads per library) and MAGs were recovered using the MetaWRAP workflow.

A total of 13 taxonomic classifications were obtained from all sub-communities (11 up to species level 1 to family level and 1 to genus level) from 26 recovered MAGs. *Escherichia coli* and *Sphingobacterium* sp. were present in all groups. *Acinetobacter gerveri* was only present in the outer gates and *Elizabethkingia miricola* was only found in rare gates. *Comamonas terrigena* and *Empedobacter brevis* was found in both the outer and rare gates. The remaining taxa were only found in the unsorted sub-communities. Our results support the hypothesis that separating sub-communities using cell sorting can improve the recovery of MAGs from low abundant microbial species.

---

#### 642-MEEP

Link between metabolically active soil microbiome and soil functions and their response to climate change in a grassland ecosystem

\*S. Wahdan<sup>1</sup>, W. Purahong<sup>1</sup>, F. Buscot<sup>1,2</sup>

<sup>1</sup>Helmholtz center for environmental research-UFZ, Soil ecology, Halle saale, Germany

<sup>2</sup>Leipzig university, Leipzig, Germany

Microbial communities in soil play a key role in maintaining ecosystem functions by catalyzing many processes such as organic matter breakdown and mineralization and climate regulation. Linking diversity of microbes to their contribution

to soil functions has been boosted by bar code studies using next-generation sequencing but remains challenging as it requires distinguishing metabolically active microbes within the ecosystems. In the present study, we used pair-end Illumina sequencing combined with bromodeoxyuridine (BrdU) immunocapture technique to characterize total and metabolically active bacteria and fungi in the rhizosphere soil of *Trifolium pratense* (red clover). To assess the functional capacity of the rhizosphere microbial community, we measured the activity of three microbial extracellular enzymes ( $\beta$ -glucopyranosidase, N-acetyl-  $\beta$ -glucosaminidase and phosphatase), which play a central role in the C, N and P acquisitions in soil ecosystem. The study was conducted on the Global Change Experimental Facility (GCEF) that simulates a scenario of future climate based on several models of climate change in Central Germany for the years between 2070 and 2100. We estimated that 42.8 % of bacteria and 32.1 % of fungi in soil are metabolically active. We also found that active bacterial richness significantly positively correlates with  $\beta$ -glucosidase activity and with N-acetyl-glucosaminidase activity ( $P < 0.05$ ) while active fungal richness significantly positively correlates with phosphatase activity ( $P < 0.05$ ). On the other hand, total microbial richness had no significant correlation ( $P > 0.05$ ) with any enzyme activities. We emphasis on considering the active microbiome to study the relationship between biodiversity-ecosystem functioning.

---

#### 643-MEEP

**Characterization of 2-phenanthroate-CoA ligase**

\*I. Kaplieva-Dudek<sup>1</sup>, R. U. Meckenstock<sup>1</sup>

<sup>1</sup>Universität Duisburg-Essen, Biofilm Center (Aquatische Mikrobiologie), Essen, Germany

**Introduction:** In anaerobic degradation of aromatic hydrocarbons, the formation of the corresponding CoA thioester at the carboxyl group is crucial for the further degradation pathway. It is known that CoA ligases are oxygen insensitive and require ATP to bind CoA to the carboxylic group. However, little is known about the CoA ligases involved in the thioesterification of PAHs, especially the one present in the novel sulfate-reducing enrichment culture TRIP, which can convert 2-phenanthrenecarboxylic acid to 2-phenanthroyl-CoA.

**Objectives:** In this work, an attempt is made to investigate the 2-phenanthroate-CoA ligase from TRIP culture. The aim is to characterize substrate specificity, the effect of other nucleotides and cations on the activity. Moreover, three genes encoding putative phenanthroate-CoA ligase from TRIP1 are planned to be heterologously expressed in *E.coli* and proofed in their activity. **Materials & Methods:** For enzymatic assays, crude cell extract of TRIP culture is used with 2-phenanthrenecarboxylic acid as control substrate and MOPS/KOH (100 mM, pH 7.3) with 15 mM MgCl<sub>2</sub> as standard buffer. To characterize substrate specificity several other aromatic hydrocarbon acid are tested. Furthermore, various cations instead of Mg<sup>2+</sup> as well as other nucleotides instead of ATP are added into the standard enzyme assay.

**Results:** 2-phenanthroate-CoA ligase is able to convert not only 2-phenanthroic acid to its corresponding CoA thioester, but also some other aromatic hydrocarbon acid. However, the enzyme activity was lower compared to the control substrate. The use of nucleotides other than ATP as well as cations other than Mg<sup>2+</sup> affects the ligase activity.

**Conclusion:** 2-phenanthroate-CoA ligase is not substrate specific and shows similarities in their activity to already known CoA ligases.

---

## 644-MEEP

### Response of a methane-driven interactome to stress intensification

\*A. Ho<sup>1</sup>, L. Mendes<sup>2</sup>, H. J. Lee<sup>3</sup>, T. Kaupper<sup>1</sup>, Z. Jia<sup>4</sup>, M. A. Horn<sup>1</sup>

<sup>1</sup>Leibniz University Hannover, Institute for Microbiology, Hannover, Germany

<sup>2</sup>University of São Paulo-USP, Brazil, Center of Nuclear Energy and Agriculture, São Paulo, Brazil

<sup>3</sup>Kunsan National University, Department of Biology, Gunsan, South Korea

<sup>4</sup>Chinese Academy of Sciences, Institute of Soil Science, Nanjing City, China

Methanotrophs rarely live in seclusion, and may select for specific interacting partners, forming a methanotroph interactome. The methanotroph interactome that comprises of methanotrophs and non-methanotrophs, is thought to modulate methane oxidation and give rise to emergent properties beneficial for the methanotrophs. Therefore, the interactome may become relevant for community functioning under stress. Here, we determined the response of a methane-driven (incubation under ~2%v/v methane) interactome to a step-wise increase in NH<sub>4</sub>Cl-induced stress (0.5 to 4.75 g N L<sup>-1</sup>, in 0.25 – 0.5 g N L<sup>-1</sup> increments) using oxic soil microcosm incubations. Incubations without supplemental ammonium served as reference. Although ammonium and intermediates of ammonium oxidation are known to inhibit methane oxidation, methanotrophic activity was unexpectedly detected even in incubations supplemented with high ammonium levels, albeit rates were significantly reduced. Both the 16S rRNA and *pmoA* (structural gene encoding for the methane monooxygenase) gene sequence analyses revealed divergent communities in the reference and stressed incubations. The 16S rRNA-based co-occurrence network analysis performed to explore potential interactions within the methanotroph interactome, revealed a more complex and modular community structure in the reference incubation, likely driven by more stable and predictable interactions when compared to the stressed community. Interestingly, the non-methanotrophs formed the key nodes, and appear to become more important with intensified stress. Extrapolating to a wider context, stress intensification unravels the interaction network, with adverse consequences for community functioning.

---

## 645-MEEP

### Response of an active methane-oxidizing community to desiccation-rewetting.

\*T. Kaupper<sup>1</sup>, M. A. Horn<sup>1</sup>, A. Ho<sup>1</sup>

<sup>1</sup>Leibniz University Hannover, Institute of Microbiology, Hannover, Germany

Desiccation - re-wetting is a recurring phenomenon in rice paddies exerting an impact on the soil microbial community. Rice paddy soils are drained bi-annually during rice harvest and re-flooded with the start of the new planting season. Since rice paddy soils contribute substantially to global methane emission, it is relevant to determine the methane-oxidizing microbial community (i.e., both the methane-oxidizing bacteria, MOB, as well as other microorganisms associated to the MOB) and their resistance / resilience to desiccation - re-wetting. It is known that MOB form a close network with their interacting partners but it is barely understood how these react in response to desiccation. To relate key microorganisms to community functioning, a stable-isotope probing (SIP) experiment was performed using a rice paddy soil to determine the impact of desiccation - re-wetting on the active methane-oxidizing community. Methane oxidation was monitored, using gas chromatography (GC), while the methanotrophic community

abundance was followed via group specific qPCR assays targeting the MOB subgroups type Ia, Ib and II. DNA-based SIP was coupled to MiSeq sequence analysis to determine the change in the active methane-oxidizing community after desiccation - re-wetting. The disturbance adversely affected methane uptake rate only in the short term. Thereafter the trend in methane uptake was generally comparable in the un-disturbed and disturbed incubations. A dominance of type I over type II MOB was observed during the incubation, showing community resilience to the disturbance. The abundance of the more oligotrophic type II MOB increased after desiccation in the later stages of incubation, when nutrients became limiting. A shift in the active microbial community composition was detected after desiccation indicated by a change in the abundance of the community members, and resulting in a less diverse but more connected network. Altogether, our results showed that MOB are resilient to single desiccation events given sufficient recovery periods, even though their associated microbial community may change.

---

## 646-MEEP

### PredicTF: a tool to predict bacterial transcription factors in complex microbial communities

L. M. Oliveira Monteiro<sup>1,2</sup>, J. P. Saraiva<sup>1</sup>, R. Brizola Toscan<sup>1</sup>, M. Gomes<sup>3</sup>, Z. Human<sup>4</sup>, P. Baldrian<sup>4</sup>, R. Silva-Rocha<sup>2</sup>, \*U. Nunes da Rocha<sup>1</sup>

<sup>1</sup>Helmholtz Center for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany

<sup>2</sup>Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

<sup>3</sup>University of Minho, Braga, Portugal

<sup>4</sup>Institute of Microbiology of the Czech Academy of Sciences, Prague, Czech Republic

Transcription factors (TF) are proteins controlling the rate of transcription of genetic information, regulating cellular gene expression. A better understanding of TF in a microbial community context will open revenues for exploring gene regulation in ecosystems where microbes are key players. To the best of our knowledge, no platform supports prediction and classification of putative bacterial TF from "omics data recovered from microbial communities. This study describes the development of PredicTF, a tool to predict TF and their families from "omics data.

First, we collected publicly available data on TF. Initially, we chose to collect data from CollecTF; a bacterial TF database containing 390 experimentally validated TF distributed over 44 TF families. Later, we retrieved TF sequences directly from Uniprot for bacterial TF Reviewed (Swiss-Prot) and manually annotated. The TF sequences were merged generating a robust TF database with 11.961 TF sequences. This database was used to train a deep learning model to predict new TF and their families. Five model organisms (*Escherichia coli*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Azotobacter vinelandii* and *Caulobacter crescentus*) were used to test the performance and the accuracy of PredicTF.

Based on our TF database, PredicTF was able to identify 35.40%, 27.23%, 51.19% 60.53% and 38.04% of the known TF with accuracy of 88.51%, 73.91%, 91.43%, 90.40% and 83.93% for the selected 5 model organisms respectively. Further, putative annotated TF in the genome of *E. coli* and *B. subtilis* were identified with accuracy of 85.71% and 100%.

We will further evaluate PredicTF using a two steps approach. First, we will test PredicTF with 8 bacterial species isolated from environmental samples from which

genomes and transcriptomes are available. After, we will determine the efficiency of PredictTF in complex microbial communities from forest soils from which metagenomes and metatranscriptomes have been sequenced.

This approach will provide the first look at TF distribution and it opens the potential to evaluate regulatory networks in complex microbial communities.

---

#### 647-MEEP

The author has not agreed to a publication.

---

#### 648-MEEP

##### Phytoplankton traits shift along gradients in species-specific ways

\*P. Hofmann<sup>1,2</sup>, A. Chatzinotas<sup>1,3</sup>, S. W. Harpole<sup>2,3,4</sup>, S. Dunker<sup>2,3</sup>

<sup>1</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Microbiology - UMB, Leipzig, Germany

<sup>2</sup>Helmholtz Centre for Environmental Research - UFZ, Department of Physiological Diversity - PhyDiv, Leipzig, Germany

<sup>3</sup>Deutsches Zentrum für integrative Biodiversitätsforschung – iDiv, Leipzig, Germany

<sup>4</sup>Martin-Luther-Universität Halle-Wittenberg, Halle, Germany

#### Question

How do traits of phytoplankton strains differ along gradients of temperature and resource supply ratio?

#### Methods

Short-term experiments inoculated with six phytoplankton strains, including three green algae and three cyanobacteria in a climate cabinet. The microcosms were exposed to constant light conditions at different temperature levels (15 - 30°C) and nitrogen : phosphorus ratios (5:1,20:1,35:1,80:1) for two weeks. Growth was measured daily with a plate reader, while single-cell traits (cell volume and chlorophyll a fluorescence) were monitored with an image-based flow cytometer.

#### Results

Overall, Temperature was affecting trait shifts more strongly than resource supply ratio, although these shifts in cell volume and chlorophyll a fluorescence were not unidirectional for all strains and traits did not respond uniformly to the environmental gradients.

For the selected strains, shifts in traits along these gradients were not shared according to the phylogenetic relationships, but rather specific to each species. Furthermore the shifts in single cell volume distributions depending on the environmental conditions carry implications for associated functions, such as sinking rate, surface area to volume ratio and likelihood of predation and by extension survival of individuals.

#### Conclusions

Cellular volume and chlorophyll a fluorescence both shifted according to temperature and resource supply ratio. Species responded differently to these gradients and their responses were not mirrored by their phylogenetic relationships. Insight into trait shifts related to the environment could potentially improve understanding of patterns within phytoplankton communities within and across years as well as in response to future conditions.

---

#### 649-MEEP

##### Diversity of anaerobic methanotrophic communities in deep sediments of Lake Baikal

\*A. Lomakina<sup>1</sup>, T. Pogodaeva<sup>1</sup>, O. Shubenkova<sup>1</sup>, V. Ivanov<sup>1</sup>, I. Khal'zov<sup>1</sup>, T. Zemskaya<sup>1</sup>

<sup>1</sup>Limnological Institute, Irkutsk, Russian Federation

Lake Baikal is the world's only freshwater reservoir where gas hydrates (GH) are found, and it describes more than 50 geological structures that differ in the composition of the discharged fluids (Khlystov et al., 2018, 2019). Process of AOM in deep sediments of Lake Baikal was revealed by radioisotope techniques, with the maximum that was usually close to the GH layers, as well as by the methane profile (Zemskaya et al., 2008; 2010). Methane concentrations in the sediments at the investigated sites increase with depth, reaching the maximum values, and the obtained profiles of methane distribution curves are similar to those in the AOM zones of other freshwater ecosystems (Schubert et al., 2011; Weber et al., 2017). It is known that electron acceptors at AOM can be not only sulfate ions (concentrations which in deep sediments of Lake Baikal ranged from 5.2 µM to 15000 µM), but also oxidized forms of Fe and Mn, as well as nitrate and nitrite ions. In deep sediments of Lake Baikal dominated reduced forms of Fe (up to 100 µM), and nitrate and nitrite ion concentrations did not exceed 9.6 µM. We have shown that in sediments of the discharge areas, located in the South and Central of Lake Baikal, members of nitrate and nitrite-dependent AOM (archaea ANME-2d and bacteria NC10) (Lomakina et al., 2018a) have been detected by molecular biology methods. Using methods of fluorescent in situ hybridization of anaerobic methanotrophic microorganisms were detected in the enrichment cultures from bottom sediments of a mud volcano Peschanka and the Krasniy Yar seepage area. In the enrichment cultures under anaerobic cultivation, methane content in the gas phase decreased, and microbial consortia were established (Lomakina et al., 2018b). The presence of genes of AOM (*mcrA* of ANME-2d subcluster *η* *pmoA* of phylum NC10) was established in the enrichment cultures from bottom sediments of the Krasniy Yar seepage area.

The work was carried out within the Russian Foundation for Basic Research (grant # 18-04-00244\_a) and the framework of the State Assignment, topic no. 0345–2019–0007.

---

#### 650-MEEP

The author has not agreed to a publication.

---

#### 652-MEEP

##### Linking the leaf microbiome to leaf metabolite landscape

\*M. Murillo-Ros<sup>1</sup>, H. S. M. Abdullah<sup>1</sup>, N. Ueberschar<sup>2</sup>, M. Agler<sup>1</sup>

<sup>1</sup>Friedrich-Schiller-Universität, Plant Microbiome Lab - Department of Microbiology, Jena, Germany

<sup>2</sup>Friedrich-Schiller-Universität, Mass Spectrometry Platform, Jena, Germany

#### Introduction:

In recent years there has been an increasing interest to study how the phyllosphere microbiome assembles. Understanding how this occurs in the inter-cellular space of the leaf (e.g. the apoplast), would provide major insights on plant-microbe interactions. This environment is truly dynamic; the fluctuation of nutrients and a large array of defense molecules, suggests it is a challenging niche for microbial colonization, where microbe-microbe interactions may play an important role. Currently, characterizing

microbial activity in the plant requires huge technical replication to overcome host signals, making it practically impossible in realistic scenarios.

## Objective:

We aim to develop a method to simultaneously obtain a snapshot of host physiology and microbial diversity in the apoplast, allowing researchers to better understand the dynamics of plant-microbe interactions under natural conditions.

## Materials and methods:

We tested an infiltration-centrifugation technique to recover apoplastic fluid (AFW) from leaves of five diverse plant species. AFW from single leaves were then split, with one fraction utilized for untargeted metabolomics and the other for studying diversity or functions of associated microbiota. We optimized buffers and conditions to eliminate cytoplasm leakage in problematic leaves of *Flaveria*. The efficiency of recovery of microbial diversity in the AFW fractions vs. in whole leaves is being tested using amplicon sequencing.

## Results and outlook:

Infiltration using sodium phosphate buffer (100 mM, pH 6.5), followed by centrifugation at 2500 x g for three minutes allowed the recovery of approximately 0.14 g AFW/ g fresh leaf for *Flaveria robusta*. This approach appears to be applicable to most plant species we tested since cytoplasm-specific metabolites were not detected. We observed species-specific amino acid profiles in the different plants, which we hypothesize are relevant for microbial nutrition. Additionally, PCR of 16S rRNA genes in AFW show clear bacterial signals with low host DNA contamination. Thus, we are now able to assess microbial diversity and activity simultaneously with host physiology.

## 653-MEEP

### Red Queens in and below the Goldilocks zone

R. Jerdan<sup>1</sup>, \*A. Spiers<sup>1</sup>

<sup>1</sup>Abertay University, School of Applied Sciences, Dundee, United Kingdom

Many *Pseudomonas* spp. are able to form air-liquid (A-L) interface biofilms in static liquid microcosms, and the eco-evolutionary dynamics of the model *P. fluorescens* SBW25 system are well-understood. In particular, adaptive lineages such as the Wrinkly Spreader gain a fitness advantage through accessing the high-O<sub>2</sub> region of the microcosm, known as the Goldilocks zone, by biofilm-formation. Just like the Red Queen, Wrinkly Spreaders are constantly competing for O<sub>2</sub> and further adaptation can occur within the biofilm itself. We have begun to extend our understanding of biofilm-formation to community-based biofilms using soil-wash inocula dominated by biofilm-competent pseudomonads. Communities were subject to serial transfers with different selection regimes including incubation conditions, sample type and transfer times, and community growth, biofilm strength and attachment levels were assessed for each transfer and for randomly-chosen isolates recovered from the initial and final-transfer communities. Although the communities under selection did not produce increasingly-strong biofilms dominated by a few "super-biofilm" strains as expected, a significant loss of diversity was observed, and a phenotypic shift occurred between the initial and final selected communities, demonstrating that these communities were in fact subject to selection. Substantial growth was also

observed under the biofilms, and tests of communities and isolates suggests that substantial productivity could occur in the low-O<sub>2</sub> region of these microcosms as well as in the Goldilocks zone. It is possible that biofilm-competent strains might avoid competition at the A-L interface by choosing a less competitive niche lower down the liquid column in a biochemical trade-off in which lower growth rates resulting from O<sub>2</sub>-limitation are balanced by the cost of biofilm-formation which would have been required at the A-L interface.

## 654-MEEP

Microbial colonization of plants at different developmental stages leads to diverse plant phenotypes and survival rates.

\*T. Mayer<sup>1</sup>, P. Baumann<sup>1</sup>, M. Reichelt<sup>2</sup>, J. Gershenzon<sup>2</sup>, M. Agler<sup>1</sup>

<sup>1</sup>Friedrich-Schiller Universität Jena, Microbiology, Jena, Germany

<sup>2</sup>Max Planck Institute for Chemical Ecology, Biochemistry, Jena, Germany

## Introduction

Variation is omnipresent in nature and plays an important role in evolution. Plants of the same genotype usually show various phenotypes, including different susceptibility to microbial pathogens, but the role plant-associated microorganisms play in generating variation is still unclear. These microbes interact with each other and the plant in complex networks which underlying mechanisms as well as their impact on other species we poorly understand.

## Objective

We aim to find out how plant-associated microbial community assembly affects variation by interrupting the natural colonization process in different plant populations from a small geographical scale to reveal population-specific differences and local adaptations of the plants microbiota.

## Material & Methods

We collected wild *Arabidopsis thaliana* populations in Jena, Germany and characterized their diversity using length polymorphisms of three microsatellite loci, a flowering time assay and analysis of leaf aliphatic glucosinolate profiles. We isolated bacteria from two of these populations using traditional culture techniques and a new *in-planta* isolation approach. In addition, we performed time-series experiments disturbing the natural colonization.

## Results

We identified four wild *A.thaliana* populations with very low intra- and high inter-population plant heterogeneity and differential culturable microbial diversity. *In-planta* enrichment of microbes suggests that a minority of leaf-associated bacterial taxa can colonize these plants. Wild plants treated with a common inoculum at different developmental stages had lower survival rates and supposedly higher size variation by similar average size than uninoculated plants. In addition, survivors were more resistant to biotic stress. This effect was not found in the *A.thaliana* ecotype Col-0.

## Conclusions

We hypothesize that amongst other things variation is caused by stochastic early colonization depending on the genotype and the environment. Hereby, the first colonizers

influence survival and phenotypic variation of their host as well as the establishment of the microbial community by microbe-plant and microbe-microbe interactions.

---

## 655-MEEP

Cross feeding among plant endophytic bacteria and its role in plant resilience to pathogens

\*A. T. Odejide<sup>1</sup>, H. S. M. Abdullah<sup>1</sup>, M. Murillo-Roos<sup>1</sup>, N. Ueberschär<sup>2</sup>, M. Agler<sup>1</sup>

<sup>1</sup>Friedrich-Schiller-Universität, Plant Microbiosis Lab - Department of Microbiology, Jena, Germany

<sup>2</sup>Friedrich-Schiller-Universität, Mass Spectrometry Platform, Jena, Germany

### Introduction:

Microbial communities play important roles in the health of plants. However, recent evidence has shown leaf-endophytic bacteria reprogram their metabolism upon entering the leaf and alter the chemical landscape to thrive, suggesting it is a difficult niche to colonize. Metabolic cross-feeding may enable auxotrophic microbes to survive in such an environment. Upon a pathogen infection, new niches are created, possibly altering the abundance of these microbes. We are interested in understanding whether this is relevant for the fitness of the plant.

### Objective:

We aim to understand whether the survival of endophytic auxotrophs, through cross-feeding, plays a role in resilience of plants to pathogens.

### Materials and methods:

We enriched and isolated phyllosphere bacteria from *Flaveria trinerva* and *F. robusta* and tested their carbon consuming strategies. We then checked if their growth was enhanced by cross-feeding with other microbes. By untargeted metabolomics, we characterized the amino acid profile in the apoplast of healthy plants. To test the relevance of these interactions on the fitness of the plant, synthetic communities of endophyte microbes, isolated from healthy and diseased leaves, will be confronted to a fungal pathogen *in planta*.

### Results and outlook:

We found that the isolated bacteria have different carbon-metabolizing strategies and that cross-feeding favours their survival in minimal media. We also discovered higher microbial load in diseased leaves, than in healthy samples. Differences in the amino acid profile of the apoplast from these two species suggest microbe-microbe interactions and their relevance in plant, may be influenced by the availability of nutrients in the environment.

---

## 656-MEEP

The author has not agreed to a publication.

---

## 657-MEEP

Evolution and transmission analysis of a MDR

***M. tuberculosis*** endemic genotype in Bulgaria by whole genome sequencing

\*S. Panaiotov<sup>1</sup>, V. Tolchkov<sup>1</sup>, Y. Atanasova<sup>1</sup>, A. Baykova<sup>1</sup>, S. Yordanova<sup>1</sup>, E. Bachiyska<sup>1</sup>

<sup>1</sup>National Center of Infectious and Parasitic Diseases, Department of Microbiology, Sofia, Bulgaria

### Introduction

An outbreak of multidrug-resistant tuberculosis was detected in Bulgaria in 2008 when spoligo and MIRU-VNTR genotyping was introduced. Molecular typing confirmed strong association between MDR and SIT41 (TUR) MTB sublineage. The MDR TB cases of SIT41 genotype in Bulgaria are >40%. The drug sensitive MTB strains of SIT41 genotype represented only 2%. SIT41 genotype is considered as a marker for MDR tuberculosis in Bulgaria. SIT41 (TUR) is endemic *M. tuberculosis* genotype for Bulgaria, Serbia, Albania and Turkey. The Bulgarian MIRU-VNTR profile of SIT41 (TUR) MDR *M. tuberculosis* strains is specific.

### Objectives

Our study intended to apply whole genome sequencing typing analysis to reconstruct the transmission dynamics of a MDR endemic in Bulgaria at higher resolution than 24 MIRU-VNTR analysis and to identify mutations associated with phenotypic drug resistance.

### Materials and Methods

In total, 150 MDR/XDR TB strains of SIT41 (TUR) have been characterized by spoligotyping and 24 loci MIRU-VNTR analysis. These represent about 75% coverage of the MDR/XDR TB cases of SIT41 (TUR) in the country for the period 2007-2018. Whole genome sequencing was applied for sequencing analysis of 48 SIT41 strains.

### Results

MIRU-VNTR analysis identified three clusters including 17, 26 and 67 strains. Five other clusters include between 3 and 9 strains. The largest cluster's MIRU-VNTR profile 244124132134425113333b32 was identified all over the country, suggesting a common ancestor. This genotype was not found in other countries. MIRU-VNTR analysis showed that SIT41 strains from Bulgaria differ from those from Albania, Greece, Serbia and Turkey. Epidemiological links were difficult to establish based on patients interview. Whole genome sequencing typing of 48 SIT41 strains demonstrated allelic differences between 1 and 54 alleles. Several clustered strains with identical WGS profile were identified. Identified SNP mutations with Xpert MTB/RIF assay responsible for MDR were confirmed by WGS analysis.

### Conclusions

The endemic SIT41 MIRU-VNTR MDR genotype, spreading in Bulgaria, was not found in other countries, suggesting local evolution. Contact tracing analysis could not explain how patients living at a distance of >350 km have identical MIRU-VNTR and WGS profiles.

Applying WGS we hope to date the start of this ongoing MDR endemic, to identify true transmissions across the country. The clonal expansion of SIT41 among the MDR-TB patients in Bulgaria remains to be further analyzed.

**Acknowledgements:** This project has received funding from the National Science Fund of Bulgaria GA: ДН-01-4/16.12.2016

## 658-EPP

### VSG length dependent growth rate in *Trypanosoma brucei*

\*V. Scheidt<sup>1</sup>, D. Horn<sup>1</sup>

<sup>1</sup>University of Dundee, Department of Biological Chemistry and Drug Discovery, Dundee, United Kingdom

*Trypanosoma brucei* is a eukaryotic, protist, pathogen with a parasitic life style. It causes sleeping sickness in humans and nagana in cattle in Sub-Saharan Africa. The parasite proliferates into two main forms, the procyclic form, when in the vector (tsetse fly) and the bloodstream form, when in the host. To avoid detection by the host's immune system, the parasite undergoes antigenic variation by switching its variant surface glycoprotein (VSG), which forms a coat on the cell surface. Only one VSG gene of a repertoire of thousands of different genes is expressed at the time. VSGs are expressed in one of about 20 sub-telomeric expression sites and flanked by conserved VSG specific sequences. Recent *in vivo* data showed that trypanosomes expressing short VSG genes mainly appear in an early infectious state, whereas trypanosomes expressing long VSG genes appear in late infection. This suggests that cells expressing short VSGs might grow faster than cells expressing long VSGs. To test this hypothesis the growth rate of cells expressing different VSGs is assessed *in vitro*. For that, trypanosomes, where the *Streptococcus pyogenes* CRISPR/Cas9 nuclease was integrated, were used to transfect a guide RNA upstream of the active VSG. By adding tetracycline the inducible Cas9 nuclease was expressed and a double strand break was created which led to switching to other VSGs due to the DNA repair mechanism. Cells expressing different VSGs were isolated by sub-cloning a mixed culture and used to analyse their growth by establishing growth curves and in mixing experiments. Furthermore, the VSG proteins of cells expressing three different VSGs (VSG-2, VSG-3 and VSG-6) were extracted and their oligosaccharides removed to analyse the glycosylation state. Cells expressing the short VSG-2 (1431 bp) outgrow other VSGs in a mixed culture. However, cells expressing the long VSG-6 (1590 bp) outgrow the shorter VSG-3 (1530 bp) in a mixing experiment. The glycosylation state of these three VSGs shows little difference. To analyse the growth of a high range of switched cells a mixed culture will be used to extract and sequence VSG RNA over time.

## 659-EPP

Comparative genomics of *Aspergillus fumigatus* and the influence of agriculture on ecology and azole resistance

\*A. E. Barber<sup>1,2</sup>, J. Born<sup>3</sup>, T. Sae-Ong<sup>1</sup>, G. Walther<sup>1</sup>, G. Panagiotou<sup>1</sup>, H. B. Deising<sup>3</sup>, O. Kurze<sup>1,2</sup>

<sup>1</sup>Leibniz HKI - Jena, Jena, Germany

<sup>2</sup>University of Würzburg, Würzburg, Germany

<sup>3</sup>Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

**Objectives:** *Aspergillus fumigatus* is a ubiquitous saprophyte capable of causing life-threatening invasive infections. Unfortunately, over the last decade resistance to the primary treatment, the azoles, has emerged and the dominant resistance mechanism is of environmental origin, suggesting that resistance is emerging through a selective pressure applied by the widespread usage of azoles in agriculture.

**Methods:** To examine the link between azoles in agriculture and the emergence of clinical resistance, systematic soil sampling was performed over a three year period before and after the vegetative period and azole application. We also performed WGS on 215 environmental and 50 clinical isolates.

**Results:** In total, 2875 soil samples were analyzed between 2016 and 2018. We observed a high degree of variation in the abundance of *A. fumigatus* across sites, however, fields with high *A. fumigatus* density tended to be consistently so from year to year. Strikingly, we observed a significant reduction in the abundance of *A. fumigatus* on conventional fields following azole treatment – a finding that was not repeated on an organic agriculture control field – indicating that the application of azoles is imposing a bottleneck on *A. fumigatus*. The overall resistance frequency among agricultural isolates was low, with only 1-3% of isolates from 2016-2018 showing resistance. Importantly, isolates from after the growing season and azole exposure showed a subtle, but consistent reduction in susceptibility to medical and agricultural azoles.

At the genome level, isolates from different regions and time periods did not cluster separately, indicating a lack of population structure. Comparison of environmental isolates with clinical isolates revealed several subgroups present in the environment that were not represented among clinical samples. Resistant environmental isolates were exclusively either wild type at the *cyp51a* loci or carried the TR34/L98H allele, while clinical isolates showed a much wider range of *cyp51a* mutations. Ongoing work is focused on defining fungal determinants enriched in human infection, as well as genetic changes associated with azole resistance.

## 660-EPP

Is Miconazole and Ciclopirox olamine an alternative treatment for Terbinafine-resistant *Trichophyton mentagrophytes* ITS genotype VIII?

\*F. Wittig<sup>1</sup>, S. Uhrlaß<sup>1</sup>, M. Monod<sup>2</sup>, H. Järv<sup>3</sup>, T. Simkin<sup>3</sup>, S. B. Verma<sup>4</sup>, S. Mey<sup>5</sup>, C. Sitaru<sup>6</sup>, Z. Almustafa<sup>7</sup>, A. Alhilaifawi<sup>8</sup>, H. Grob<sup>9</sup>, M. Gebhardt<sup>10</sup>, U. Krusche<sup>11</sup>, W. Hofmann<sup>12</sup>, C. Krüger<sup>1</sup>, P. Nenoff<sup>1</sup>

<sup>1</sup>Laboratory for Medical Microbiology, Partnership Prof. P. Nenoff & Dr. C. Krüger, Rötha OT Mölbis, Germany

<sup>2</sup>Centre Hospitalier Universitaire Vaudois, Dermatology Service, Lausanne, Switzerland

<sup>3</sup>SYNLAB Eesti OÜ, Tallinn, Estonia

<sup>4</sup>"Nirvan" and "In Skin" Clinics, Vadodara, India

<sup>5</sup>Preah Kossamak Hospital, Dermatology Clinic Mey Sithach, Phnom Penh, Cambodia

<sup>6</sup>University Medical Centre, Department of Dermatology, Freiburg, Germany

<sup>7</sup>Charité University Medical Centre, Department of Dermatology, Berlin, Germany

<sup>8</sup>Dermatology practice Schongau, Schongau, Germany

<sup>9</sup>Dermatology FMH, Medical practice Reinacherhof, Reinach, Switzerland

<sup>10</sup>Dermatology practice Gebhardt, Zwickau, Germany

<sup>11</sup>Dermatology practice Hradetzky/Krusche, Leipzig, Germany

<sup>12</sup>Dermatology practice Hofmann, Frankfurt am Main, Germany

Although resistance of dermatophytes against antifungals has been of minor importance for many years, the situation changed completely: primarily *Trichophyton (T.) mentagrophytes* ITS genotype VIII (Indian variant) was shown to cause chronic recalcitrant dermatophytosis associated with failing topical and oral terbinafine (TRB) treatment and *in-vitro* TRB resistance. The aim of this study was to analyze the susceptibility of TRB-resistant and -sensitive *T. mentagrophytes* VIII to miconazole (MI) and ciclopirox olamine (CIC). Results were compared to antifungal susceptibilities of *T. mentagrophytes* VII (Thai variant) and *T. rubrum*.

In total, 12 *T. mentagrophytes* VIII strains from Iraq, Finland, Cambodia and Germany, 21 *T. mentagrophytes* VII from Germany and two *T. rubrum* from Pakistan and Switzerland were included in this study. These clinically isolated strains were identified by culture, PCR-ELISA and ITS sequencing. TRB resistance was defined by growth on TRB-containing



agar and squalene epoxidase gene mutation. MIC values of MI and CIC were determined on agar.

Out of all tested *T. mentagrophytes* VIII strains 83.3%(10) were TRB-resistant, but only 16.7%(2) were TRB-sensitive. In contrast, no TRB resistance was detected in *T. mentagrophytes* VII and *T. rubrum*. While MICs of CIC were unexceptionally 8 µg/ml, MICs of MI differed between the tested *Trichophyton* genotypes and species: *T. mentagrophytes* VIII showed the lowest (<0.05-0.1 µg/ml) and *T. mentagrophytes* VII the highest (0.4 µg/ml) MIC value. In between, there was the MIC value (0.2 µg/ml) of *T. rubrum*.

As recently described by Nenoff *et al.*(2019), a six-month-old female patient from Bahrain suffering from an extensive dermatophytosis caused by a TRB-resistant *T. mentagrophytes* VIII strain was successfully treated with topical MI and CIC. In this study, we show for the first time that *T. mentagrophytes* VIII strains are highly susceptible to MI *in-vitro*, even higher than *T. mentagrophytes* VII and *T. rubrum*. As TRB resistance and the geographical origin of the patient did most likely not influence the susceptibility to MI and CIC, we suggest both antifungals as an efficient topical alternative treatment.

#### 661-EPP

Using *in vitro* and *ex vivo* models to unravel *Candida auris* infection strategies

\*S. Allert<sup>1</sup>, D. Schulz<sup>1</sup>, P. Kämmer<sup>1</sup>, P. Großmann<sup>2</sup>, T. Wolf<sup>2</sup>, S. Brunke<sup>1</sup>, B. Hube<sup>1</sup>

<sup>1</sup>Hans-Knoell-Institute, Microbial Pathogenicity Mechanisms, Jena, Germany

<sup>2</sup>Hans-Knoell-Institute, Systems Biology and Bioinformatics, Jena, Germany

*Candida* species are a major cause of invasive fungal infections, with *Candida albicans*, *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* being the clinically most relevant ones. Recently, *C. auris* emerged as a new species affecting humans in various regions of the world, causing invasive infections with high rates of clinical treatment failures and problems with nosocomial transmission.

As the pathogenicity mechanisms of *C. auris* are largely unknown, we analyzed a set of *C. auris* strains from different clades during interaction with host cells and *in vitro* under conditions mimicking the host or environmental situation. Although the different strains were variable in drug resistance, aggregation and other *in vitro* growth phenotypes, the overall behavior in our infection models was similar. *C. auris* strains did not cause severe damage of epithelial cells, in contrast to *C. albicans*. Human macrophages rapidly phagocytose *C. auris* cells and respond by distinct cytokine secretion. However, *C. auris* can survive inside of macrophages and shows intracellular replication comparable to *C. glabrata*. To mimic the initial phase of systemic *Candida* infections with dissemination via the bloodstream, we used an *ex vivo* whole blood infection model. Similar to *C. albicans* and *C. glabrata*, *C. auris* is efficiently killed in human blood and shows a species-specific pattern in immune cell association, survival rate and induction of cytokines. Dual transcriptional profiling of *C. albicans*, *C. parapsilosis*, *C. tropicalis* or *C. glabrata* and blood cells during infection indicates discrete survival strategies of all four fungal species, while the transcriptional host response is rather uniform. Follow-up RNA-sequencing analysis of *C. auris*-infected blood has given first insights. For instance, in line with the observations for the other four *Candida* species, the human response to *C. auris* in blood is governed by an upregulation of pro-inflammatory cytokines

and chemokines. Further analysis of the transcriptional profiling data will help us to place *C. auris* within the pathogenic landscape of *Candida* species.

#### 662-EPP

Gut microbiota-mediated colonization resistance against *Candida albicans*

\*W. Krüger<sup>1</sup>, S. Vielreicher<sup>1</sup>, R. Santhanam<sup>2</sup>, M. Stock<sup>3</sup>, M. J. Niemiec<sup>1</sup>, I. D. Jacobsen<sup>1</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology, Microbial Immunology, Jena, Germany

<sup>2</sup>Leibniz Institute for Natural Product Research and Infection Biology, Systems Biology and Bioinformatics, Jena, Germany

<sup>3</sup>Centre for Innovation Competence Septomics, Host Septomics, Jena, Germany

The human gut provides a niche for various microbes, including fungi. The fungus *Candida albicans* is commonly found on mucosal surfaces of healthy individuals, but can also cause superficial to disseminated infections. Microbial dysbiosis can increase fungal proliferation and lead to mucosal and systemic candidiasis. Over the past decades, incidence with *C. albicans* has increased in intensive care units. Understanding which intestinal microbes control this commensal might contribute to prevention of the disease.

The aim of this project is to examine the influence of the intestinal microbiota composition on *C. albicans* colonization and to study the impact on the host immune response.

Genetically identical mice were analyzed for differences in their fecal microbes by microbiome analysis and colonized with *C. albicans*. To investigate the effect of several bacterial groups, different antibiotic treatments were implemented prior to *C. albicans* inoculation. Changes in bacterial burden were measured by CFUs at several time points during the experiment. Flow cytometry was used to investigate effects on immune cells in the spleen and Peyer's patches.

Analysis revealed variations in fungal and bacterial composition in genetically identical mice from different breeders, while they showed similar *C. albicans* colonization rates 14 days after inoculation. In contradiction to the general assumption that adult laboratory mice are naturally resistant to *C. albicans* colonization (Koh, 2013; Prieto *et al.*, 2014), we detected up to  $1 \times 10^5$  CFUs/g in fecal samples and intestinal organs in the absence of antibiotic treatment. However, depletion of intestinal bacteria by antibiotics enhanced colonization in all mice. Additionally, organs of untreated mice of different colonies varied in their immune composition and showed differential immune reactions to *C. albicans* colonization and bacterial depletion.

Thus, despite variation in the microbial composition, and in contrast to previous reports by others, genetically identical mice could be colonized with *C. albicans* at comparable rates, with antibiotic-induced dysbiosis increasing colonization levels.

#### 663-EPP

The peroxiredoxin Asp f3 is crucial for redox balance, sensing of reactive oxygen species and virulence in *Aspergillus fumigatus*

\*J. M. Boysen<sup>1,2</sup>, N. Saeed<sup>1,2</sup>, E. Shekhova<sup>2,3</sup>, T. Wolf<sup>4</sup>, G. Panagiotou<sup>4</sup>, O. Kniemeyer<sup>3</sup>, F. Hillmann<sup>1</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Evolution of Microbial Interactions, Jena, Germany

<sup>2</sup>Friedrich-Schiller-University, Jena, Germany

*Aspergillus fumigatus* is a filamentous ascomycete of saprophytic lifestyle which is also known as an opportunistic pathogen. The highest risk to develop *A. fumigatus* derived infections, ranging from allergic reactions to often fatal invasive aspergillosis, lies with immunocompromised patients suffering from conditions such as leukemia, organ or stem cell transplantation, HIV or chronic granulomatous disease. How *A. fumigatus* adapts its metabolic needs to the host environment of the lung is a central question in its infection biology. We have previously identified the major allergen Asp f3 to act as a functional 2-Cysteine-peroxiredoxin that plays an essential role for invasive growth in a mouse model for aspergillosis, demonstrating that the fungus depends on a functional redox homeostasis during growth in the host. The deletion mutant of *asp f3* grows wildtype-like unless challenged with ROS. The hypersensitivity to ROS was found to be strictly dependent on both conserved cysteine residues of the protein. To elucidate the role of Asp f3 in the redox homeostasis of the fungus during ROS exposure, we took a redox proteomics approach to compare the global proteins oxidation state in the wild type and the  $\Delta$ asp f3 strain following hydrogen peroxide treatment. Thus we gained insight into the targets of ROS associated damage and could identify proteins with proposed extracellular functions in protein folding as well as central metabolic enzymes to be stronger oxidized in  $\Delta$ asp f3. We also established an *in vivo* assay, which allows specific exposure to external pulses of superoxide (O<sub>2</sub><sup>-</sup>), the primary product of the NADPH oxidase in cells of the innate immunity, to monitor the transcriptional changes in *A. fumigatus* confronted with ROS. RNASeq analysis revealed identical transcriptomes for unchallenged wildtype and  $\Delta$ asp f3. However, in the presence of external ROS the mutant displayed a deregulation of the fungal oxidative stress response, leading to a number of differentially expressed genes which are for example part of oxidation-reduction processes, transmembrane transport or gliotoxin biosynthesis.

#### 664-EPP

Living inside the host for years: the novel smut fungus *Thecaphora thlaspeos* as a model pathogen in model plants

L. Plücker<sup>1</sup>, S. Gul<sup>1</sup>, M. Feldbrügge<sup>1</sup>, \*V. Göhre<sup>1</sup>

<sup>1</sup>Heinrich-Heine University Düsseldorf, Institute for Microbiology, Düsseldorf, Germany

Smut fungi, such as *Ustilago maydis* in maize or *Thecaphora solani* in potato, are globally distributed plant pathogens that infect agriculturally important crop plants. During the major part of their life cycle, these pathogens grow as endophytes in their host plant to finally produce large amounts of fungal spores in reproductive organs. To date, the lack of genetically tractable host plants limits investigations of plant responses, and several attempts to infect *Arabidopsis thaliana* with *U. maydis* were unsuccessful. To close this gap, we have established the Brassicaceae smut fungus *T. thlaspeos* as a pathogen of model plants.

To this end, we follow three approaches. 1. Characterization of the infection biology, which has already revealed several interesting differences, e.g. root colonization previously unknown for the grass smut fungi, and an endophytic growth phase that lasts over years in perennial host plants (1).

Currently, we are following up on local plant responses to infection using plant and fungal reporter lines. 2. Establishing the fungus as a model organism comparable to *U. maydis*. This included cultivation, genetic manipulation, and sequencing the genome. Notably, in contrast to the dimorphic smut fungi, *T. thlaspeos* growth exclusively in a filamentous form, but still has a typical smut genome with the conserved *a* and *b* mating locus (2). 3. Characterization of protein and RNA virulence factors to identify conserved and specific virulence factors. Interestingly, the effector repertoire differs from grass smut fungi, and our *Thecaphora*-specific top candidate effector links infection to plant cold acclimation. Here, I will report on our latest findings on the interaction between *T. thlaspeos* with its host plants in this novel model pathosystem.

1. L. Frantzeskakis *et al.*, The plant-dependent life cycle of *Thecaphora thlaspeos*: a smut fungus adapted to Brassicaceae. *Mol Plant Microbe In* **30**, 271-282 (2017).
2. K. J. Courville *et al.*, Smut infection of perennial hosts: the genome and the transcriptome of the Brassicaceae smut fungus *Thecaphora thlaspeos* reveal functionally conserved and novel effectors. *New Phytologist* **222**, 1474-1492 (2019).

#### 665-EPP

##### Functional Analysis of the *Candida albicans* *ECE1* promoter

\*R. Martin<sup>1,2</sup>, O. Kurzej<sup>1,3,2</sup>

<sup>1</sup>University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany

<sup>2</sup>Leibniz Institute for Natural Product Research and Infection Biology- Hans Knoell Institute-, National Reference Center for Invasive Fungal Infections, Jena, Germany

<sup>3</sup>Leibniz Institute for Natural Product Research and Infection Biology- Hans Knoell Institute-, Research Group Fungal Septomics, Jena, Germany

#### Introduction

*ECE1* is normally the most abundant transcript in *C. albicans* hyphae, but barely detectable in yeast cells. It was recently shown that the Ece1 protein is processed into different peptides which are secreted from the hyphae into the environment. One of these peptides, Candidalysin, is cytolytic and contributes to damage of host cell membranes. Therefore, a better understanding of the regulation of this gene will help to better understand fungal virulence.

#### Objectives

The aims of this study were to identify the TATA box required for the transcription of the gene, to determine the length of the actual *ECE1* promoter and to examine if different hyphal growth stimulators can induce alternative routes of transcription.

#### Material and Methods

To address the objectives of the study, we used RNAseq data and RACE PCR to identify the 5' untranslated region (UTR) of *ECE1*. In a next step, we performed site-directed mutagenesis in a construct where GFP is under control of the *ECE1* promoter to perform a functional analysis of putative TATA boxes. To determine the length of the promoter, truncated versions of it were fused to GFP and ectopically integrated into the *C. albicans* *NEUT5L* locus.

Consequently, the resulting mutants were screened for GFP signal intensities after the induction of hyphal growth.

## Results

The size of the 5'UTR was always 50bp, independent from the stimulus used to trigger hyphal growth. In accordance, a TATA box which is 109bp upstream of the start codon was identified as the one required for the start of transcription. The GFP screening with truncated versions of the 5' intergenic region showed that the minimum size of the promoter was between 1000 and 1500bp.

## Conclusions

Within this study we identified the TATA box required for the transcription of *ECE1* and determined the length of the promoter. Our results show that a region 1000-1500bp upstream of the *ECE1* start codon is required for the expression of the gene. This active region contains putative binding sites for several transcription factors, indicating that an intense regulation controls the transcription of *ECE1* in the different morphologies of *C. albicans*.

---

### 666-EPP

#### Analysis of the intestinal mycobiome composition in a non-alcoholic fatty liver disease patient cohort

\*N. Thielemann<sup>1</sup>, M. Rau<sup>2</sup>, S. Leal Siliceo<sup>3</sup>, M. Herz<sup>1</sup>, H. Hermanns<sup>2</sup>, R. Martin<sup>1,4</sup>, G. Panagiotou<sup>3</sup>, A. Geier<sup>2</sup>, O. Kurzai<sup>1,4,5</sup>

<sup>1</sup>University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany

<sup>2</sup>University Hospital Würzburg, Department of Medicine II, Division of Hepatology, Würzburg, Germany

<sup>3</sup>Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute, Research Group Systems Biology and Bioinformatics, Jena, Germany

<sup>4</sup>Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute-, National Reference Center for Invasive Fungal Infections, Jena, Germany

<sup>5</sup>Leibniz Institute for Natural Product Research and Infection Biology-Hans Knoell Institute-, Research Group Fungal Septomycosis, Jena, Germany

## Introduction

The prevalence of the emerging non-alcoholic fatty liver disease (NAFLD) is estimated to be around 25-35% in Europe. NAFLD pathogenesis is characterized by excessive accumulation of fat in hepatocytes, constituting a non-alcoholic fatty liver (NAFL) in early disease stages, which could develop to non-alcoholic steatohepatitis (NASH) in advanced disease stages. As the liver is closely connected to the gut via portal vein blood supply, it is exposed to high fat diet-related implications like intestinal microbiome composition shifts. It has been shown before, that in advanced NAFLD stages the bacterial composition of the intestinal microbiome significantly changes in comparison to early disease stages and controls. However, less is known about the intestinal mycobiome in NAFLD pathogenesis.

## Objectives

The aim of this study was to elucidate the role that intestinal fungi have in the pathogenesis of NAFLD by characterizing the gut mycobiota in NAFLD patients, using microbial DNA initially isolated and utilized for intestinal bacteriome composition analysis.

## Materials and Methods

For analysis of the intestinal mycobiome diversity in NAFLD pathogenesis, stool samples from 23 NAFLD, 14 NAFL and 18 NASH patients as well as 27 healthy controls were collected. Microbial genomic DNA was extracted from the stool samples and used for ITS1 sequencing.

## Results

ITS1 sequencing of microbial DNA samples, which were initially isolated and used for intestinal bacteriome analysis, proved successful as a wide variety of about 160 fungal species, including various *Candida* and *Aspergillus* species and *Malassezia*, were identified. We are currently analyzing the intestinal mycobiome diversity in the NAFLD disease stages in comparison to the controls.

## Conclusion

Due to the observed variety of intestinal fungi and their influence on host organism physiology, changes in the intestinal mycobiome composition of NAFLD patients compared to the controls might be detectable. As also bacteriome data are available for the NAFLD patient cohort, future work will include the correlation between mycobiome and bacteriome data and investigation of possible interaction mechanisms.

---

### 667-EPP

#### Cross-resistance between fluconazole and macrophages mediated by mitochondria function in *C. glabrata*

S. Siscar-Lewin<sup>1</sup>, T. Gabaldon<sup>2</sup>, B. Hube<sup>1,3</sup>, \*S. Brunke<sup>1</sup>

<sup>1</sup>Hans Knöll Institute, Microbial Pathogenicity Mechanisms, Jena, Germany

<sup>2</sup>Centre for Genomic Regulation (CRG), Barcelona, Spain

<sup>3</sup>Friedrich Schiller University, Jena, Germany

*Candida glabrata* has become the second most common cause of candidemia, largely due to its ability to rapidly acquire antifungal resistances. It is more closely related to the baker's yeast *Saccharomyces cerevisiae* than to other pathogenic *Candida* species, and seems to have evolved different infection strategies. While comparing its genome to closely related, nonpathogenic yeasts, we found markers of positive selection in the sequence of the gene *CgMIP1*, whose ortholog in *S. cerevisiae* codes for a mitochondrial polymerase. We investigated whether these markers are traces of *C. glabrata*'s selection in the host toward pathogenicity.

A deletion mutant of *CgMIP1* is *petite*, lacking mitochondrial function like its *S. cerevisiae* counterpart. Like the baker's yeast mutant, it also is highly fluconazole resistant, in agreement with a concomitant upregulation of efflux pumps. However, unlike the *S. cerevisiae* mutant, *C. glabrata mip1Δ* is resistant to many endoplasmic reticulum stressors, and, importantly, survives uptake by human macrophages significantly better than the wild type.

The *petite* phenotype can be triggered by passage through macrophages, as well as by continuous exposure to fluconazole. This indicates that macrophage exposure can trigger antifungal resistance – and *vice versa*, with important implications for treatment of *C. glabrata* infections. Importantly, we found *petite* phenotypes in clinical isolates which likewise better resisted both macrophages and azoles. We are currently investigating the contribution of *CgMIP1* in these resistances, but are confident that mitochondrial function connects these two clinically relevant phenotypes.

---

## 668-EPP

The castrating fungus *Microbotryum*: A parasitic genus to understand the evolution of host-specific parasitism

\*S. Klenner<sup>1</sup>, L. Engelhardt<sup>1</sup>, N. Hassel<sup>1</sup>, I. Teichert<sup>2</sup>, D. Begerow<sup>1</sup>

<sup>1</sup>Ruhr-University Bochum, Department of Geobotany, Bochum, Germany

<sup>2</sup>Ruhr-University Bochum, Allgemeine und Molekulare Botanik, Bochum, Germany

**Question:** Biotrophic fungi are characterized by intimate interactions with highly specific hosts and often cause devastating plant diseases. Biotrophic interaction necessitates an adapted set of genes to manipulate the host plant and complete the fungal life cycle. The genus *Microbotryum* comprises species of closely related fungal parasites infecting specific host plants. *Microbotryum* has a diphasic life cycle consisting of a haploid, saprotrophic sporidial stage and a dikaryotic biotrophic hyphal stage *in planta*, which interferes with flower formation of the host.

**Methods:** Bioinformatical analyses of putative host-manipulating effectors were performed to assess subcellular localization *in planta* and provide candidate genes for host-specific interaction. Therefore, we established an efficient transformation protocol to electrotransfer linear and circular DNA resulting in stable *Microbotryum* transformants to assess gene function.

**Results:** Transformation experiments show initial incubation at low temperatures is crucial for cell regeneration and formation of resistant colonies. Our results indicate that false positive rate of transformants is minimized by electroporation at 0,5 kV yielding up to  $2,5 \times 10^2$  transformants. Putative effectors were detected *in silico* and predicted to be localized in the chloroplast, plant nucleus as well as the apoplast.

**Conclusions:** Lacking efficient genetic tools to assess the role of these putative effectors, we further aim to establish a modular molecular toolbox utilizing the Golden Gate cloning strategy and our established transformation protocol. Using *BsaI* we designed specific restriction and re-ligation sites for each module and each library in our toolbox. This library includes different classes of donor plasmids, which corresponds to multiple research issues like heterologous gene expression and fluorophore tagging. In addition, it enables us to express RNAi and CRISPR/Cas9 constructs in *Microbotryum* to assess gene function.

In summary, we present two versatile and time-saving tools, which allow access not only to a single parasitic fungus, but to an entire parasitic genus to understand the evolution of host specificity.

---

## 670-FMP

Effect of pulsed light treatment on *Salmonella* Typhimurium and *Yersinia enterocolitica* reduction on pork skin and loin and influence on sensory properties

\*C. Wiacek<sup>1</sup>, F. Koch<sup>1</sup>, P. G. Braun<sup>1</sup>

<sup>1</sup>Leipzig University, Institute of Food Hygiene, Leipzig, Germany

Salmonellosis and Yersiniosis are well-known human food-borne illnesses. Both are closely associated to the consumption of raw pork meat and products thereof. Despite high hygiene standards in slaughterhouses the safety of such products cannot be ensured, which is the reason that additional decontamination measures should be taken into account. Non-thermal decontamination technologies, e.g. pulsed light (PL), are increasingly gaining interest.

Particularly in the area of minimally processed foods they can improve microbial safety. The aim of the investigations presented was to examine the bactericidal effect of PL on both bacteria on food matrices, the role of light spectra and the influence on product quality.

Typhimurium (S.T.) or *Y. enterocolitica* (Y.e.) inoculated pork loin and skin were PL-treated with a variety of different fluences and wavelength spectra by using treatment distance/time variations and bandpass and long pass filters to eliminate individual wavelength ranges.

PL treatments with the full light spectrum (200-1200 nm) resulted in significant reductions of S.T. (3.0 log) and Y.e. (3.2 log) within a few seconds. However, the sensory properties of the pork loin and skin were altered. Experiments with pork skin and various filters indicate none respectively slight reductions (0.3 log) with PL in the VIS and IR spectrum as well as in the UV-A fraction. With the addition of UV-B, reductions of 2.2 log (S.T.) and 3.0 log (Y.e.) were measurable. This means that even without UV-C, held responsible for the germicidal effect but also odor deviation, significant reductions can be achieved on pork skin. The sensory evaluation verified that previously frequently mentioned attributes "chemical" and "pungent" were used less frequently and in a more moderate manner in samples without UV-C. The perceived deviations were described as less unpleasant.

As a contact and residue free, fast and water-saving technology PL might be an interesting alternative technology for meat industry. Nonetheless, beside bactericidal effect also resulting product quality has to be taken into account because changes in odor could be an exclusion criterion for the application.

---

## 671-FMP

Plasma treated water generated by microwave-induced plasma (MidiPLexc) leads to antimicrobial effects on *Listeria monocytogenes* biofilms

\*O. Handorf<sup>1</sup>, U. Schnabel<sup>1,2</sup>, V. I. Pauker<sup>3</sup>, T. Weihe<sup>1</sup>, C. Weit<sup>3</sup>, K. Riedel<sup>3</sup>, J. Ehlbeck<sup>1</sup>

<sup>1</sup>Leibniz Institute for Plasmascience and Technology, Plasmabioengineering, Greifswald, Germany

<sup>2</sup>Environmental Sustainability and Health Institute, Food Safety and Microbiology, Dublin, Ireland

<sup>3</sup>Institute of Microbiology, Microbial Physiology and Molecular Biology, Greifswald, Germany

Plasma generated compounds (PGC's) such as plasma processed air (PPA) or plasma treated water (PTW) offer an increasingly important alternative for the treatment of microorganisms in hard-to-reach industrial applications such as those found in the food industry. Therefore, the antimicrobial effect of water treated with microwave induced plasma (MidiPLexc) on *Listeria monocytogenes* (ATCC 1513) biofilms was investigated. 10 ml deionized water was treated with the MidiPLexc plasma source (Fig. 1) for 100 s, 300 s and 900 s (pre-treatment time) and the bacterial biofilm was subsequently exposed to the PTW for 1 min, 3 min and 5 min (post-treatment time) for each treatment time. The effects of these treatments were analysed using microbial methods (CFU, fluorescence and XTT assay) and imaging techniques (fluorescence microscopy, confocal laser scanning microscopy (CLSM), atomic force microscopy (AFM)). The CFU showed a maximum reduction factor (RF) of 4.7 (300 s; 5 min), a maximum reduction in vitality of 69.5% (900 s; 5 min) (fluorescence assay) and a maximum reduction in cell metabolism of 47.9% (900 s; 5 min) (XTT assay). Fluorescence microscopy shows an increasing antimicrobial effect on biofilms with increasing pre- and post-

treatment. This was also confirmed by CLSM. The cell morphological investigations of the biofilms by AFM showed no remarkable morphological changes of the individual cells, however a change in the plasticity of the cells during the treatment could be clearly determined. This is reflected in the blurred visualization of the control biofilms compared to the treated biofilms. These properties should be confirmed by Texture-Analyser measurements as force curves. In summary, these results show a strong effect on microbial biofilms relevant for the food industry. Furthermore, the obtained data may provide new insights into the mechanisms of decontamination by PTW and thus lead to a more targeted application of plasma decontamination in theory and industry.

**Figure 1: schematic illustration of the MidiPLexc.** schematic illustration of the MidiPLexc with 1 l glas bottle attached to the bottle adapter of the plasma source.

## 672-FMP

### Change of usability of a commercial selective agar for isolation of carbapenemase-producing Enterobacteriaceae from samples of animal origin

\*N. Pauly<sup>1</sup>, J. A. Hammerl<sup>1</sup>, S. Schmoger<sup>1</sup>, M. Grobbel<sup>1</sup>, J. Fischer<sup>1</sup>, A. Käsbohrer<sup>1,2</sup>, A. Irrgang<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany

<sup>2</sup>University of Veterinary Medicine, Institute for Veterinary Public Health, Vienna, Austria

Since 2014, the European Reference Laboratory for Antimicrobial Resistances provides a protocol for isolation of carbapenemase-producing *E. coli* (CPE) from caecal and meat samples. It is used by EU member states for monitoring purposes. In our experience, the method fails to isolate CPE with slight reduced susceptibility to carbapenems. To estimate the true prevalence of CPE, a reliable isolation method is required. So, the method needs to be improved. In this process, the selective agar is a central point. Results in our lab showed differences in the detection rates depending on the age of the agar. This study aims to evaluate and compare CPE detection rate for a commonly used commercial media (ChromID® CARBA, bioMérieux) with selective and non-selective inhouse media.

To guarantee comparability, every week of our 25 week experiment, one set of lyophilisates from five CPE and two control strains were resuspended and plated on five different batches of the commercial medium as well as on LB agar, and MacConkey agar supplemented with 1mg/L cefotaxime and 0,125mg/L meropenem. After incubation for 18h at 37°C, the colony forming units (cfu) were counted. In addition, eleven participating state laboratories tested the same batch of lyophilisates on more batches of the commercial medium in the same way, but at only three timepoints.

The sensitivity of plates was determined by comparing the cfu of the CPE over the time. It seemed that the CPE with carbapenem resistances mediated by a serine-based hydrolytic mechanism was able to grow constantly over the 25 weeks. Strains harboring a carbapenemase with zinc-catalyzed activity had difficulties with growing on the commercial medium. Furthermore, the counted cfu over time showed that the detection of CPE with low MIC values increased with the age of the commercial medium. The results of the other labs confirmed ours. The selective inhouse agar proved to be more sensitive compared to the commercial.

This study shows that the commercial medium is only partially suitable for the detection of CPE with low resistances. Usage of the medium beyond the expiry date or of a homemade may increase the sensitivity of the method.

## 673-FMP

Detection and characterization of the mobile colistin resistance gene *mcr-4.3* in *Acinetobacter baumannii* recovered from wastewater treatment plants in Germany M. Savin<sup>1</sup>, M. Parcina<sup>1</sup>, J. Kreyenschmidt<sup>1</sup>, A. Käsbohrer<sup>2,3</sup>, \*J. A. Hammerl<sup>4</sup>

<sup>1</sup>University of Bonn, Faculty of Agriculture, Institute of Animal Sciences, Bonn, Germany

<sup>2</sup>German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany

<sup>3</sup>Institute for Veterinary Public Health, University of Veterinary Medicine, Wien, Austria

<sup>4</sup>Bundesinstitut für Risikobewertung, Biological Safety, Berlin, Germany

**Questions:** Antibiotics of the last resort are important for the treatment of multidrug-resistant bacteria in humans. Lately, polymyxins retained further attention as more and more mobile colistin resistance genes were identified conferring resistances against colistin in Gram-negative bacteria. Currently, epidemiological data indicated that *mcr-1* is the most prevalent colistin resistance-determinant worldwide. However, reliable data on the impact of the individual *mcr*-genes are lacking.

**Methods:** Within the HyReKa-project, ESBL-producing bacteria from process water of different stages within a poultry slaughterhouse in Germany were isolated by selective cultivation. Bacteria of the ESKAPE-group were further subjected to antimicrobial resistance-typing using the microbroth dilution method with Micronaut-S MDR MRGN-screening system.

**Results:** During this screening, an *A. baumannii* from process water of poultry eviscerators exhibiting a non-wildtype phenotype against cefotaxime, chloramphenicol, fosfomycin, colistin and intermediate sensitivity against temocillin was detected. Colistin gene-typing revealed the presence of the *mcr-4.3* gene variant. PFGE using bacteria of this *A. baumannii* isolate indicated that *mcr-4.3* is located on a 30 kb plasmid. Whole genome-sequencing indicated that the *mcr-4.3* carrying plasmid exhibits significant similarities to the previously reported *A. baumannii* plasmid. However, both plasmids differ in some regions of their plasmid genomes. *In vitro* plasmid transmission studies provide no evidence that this plasmid was transmissible to *E. coli* either by transformation nor conjugation. Furthermore, up to now we are unable to determine an intraspecies transfer of the *mcr-4.3* plasmid in *A. baumannii*.

**Conclusions:** Our study indicates that *A. baumannii* might also be a common recipient of *mcr*-carrying plasmids or involved in its dissemination. As *A. baumannii* are pathogens of high clinical importance, the acquisition of resistance determinants against last resort antibiotics forces an antimicrobial crisis that was predicted for the future without applying strict global management strategies in antibiotic usage.

## 674-FMP

### Evaluation of the toxicity of pyrrhione based Ionic Liquids

\*B. Bromberger<sup>1</sup>, J. Sommer<sup>2</sup>, C. Robben<sup>2</sup>, C. Trautner<sup>2</sup>, R. Kalb<sup>3</sup>, P. Rossmanith<sup>1,2</sup>, P. J. Mester<sup>2</sup>

## Introduction:

Over the last decades the development of new disinfection strategies gained more importance. One substance, which is already used as a potent antimicrobial is Pyrithione (2-Mercaptopyridine *N*-oxide) a derivative of the naturally occurring aspergillic acid. Pyrithione salts (mostly zinc and sodium) are widely used in shampoos, adhesives or coatings mainly functioning as bacteriostatic, fungistatic or algistatic.

**Objectives:** Even if Pyrithione is already used as an antimicrobial in different fields of application, the question comes up if its effect can be strengthened or physically modified when incorporated into an ionic liquid to open the application for new areas.

## Material & Methods:

In this study, 20 ionic liquids (ILs) with different cationic head groups and Pyrithione as anion were tested on three different biological test systems. Therefore the minimal inhibitory concentrations (MIC) of the Pyrithione-ILs were tested to determine the effects on 17 bacteria and two fungi (yeasts). Additionally the virucidal concentrations on four different corresponding virus surrogates as well as the enzyme inhibition potential via qPCR assay was tested. Beside the liquid-based test systems we conducted an agar disk diffusion test.

## Results & Discussion:

The MIC test implies that the antimicrobial effect of the pyrithione based ILs on bacteria is similarly to the pure substance. However some ILs, especially those with a long cationic side chain, had increased antimicrobial activity. Concerning the virucidal concentration short cationic side chains have almost no effect, while an increasing side chain length lead to elevated toxicity until a typical "cut-off" effect was present. On the enzymatic level similar effects could be observed, though MIC values differed based due to the sensitivity of this test method.

## Conclusion:

Overall we established ILs with pyrithione anions without losing the antimicrobial property of pyrithione. Additionally these ILs can be modified by changing properties like solubility or physical appearance.

### 675-FMP

Microbial reduction in domestic dishwashers

\*B. Brands<sup>1</sup>, S. Schulze Struchtrup<sup>2</sup>, R. Stamminger<sup>2</sup>, D. Bockmühl<sup>1</sup>

<sup>1</sup>Hochschule Rhein-Waal, Faculty of Life Sciences, Kleve, Germany

<sup>2</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Landtechnik, Sektion Haushaltstechnik, Bonn, Germany

**Introduction:** Dishwashers efficiently remove soil, bacteria and food particles, but microbial communities have been found [1, 2]. No infections via dishwashers have been detected until now. Hygiene measures like high temperature cycles reduce the microbial count in the sump of dishwashers [4]. In Europe, there are no standards to

address the hygiene in household dishwashers, whereas in the US, NSF/ANSI 184-2019, states requirements for appliances to be certified as sanitizing.

**Objectives:** Aim of this study is to develop a method that is able to determine the microbial reduction of different dishwasher cleaning cycles and differentiate between different program parameters.

**Methods:** Stainless steel biomonitors are contaminated with *Micrococcus luteus* or *Enterococcus faecium* embedded in a soil matrix (bovine serum albumin, mucin, corn starch). The logarithmic reduction (LR) on the biomonitors in different dishwashing cycles is determined.

**Results:** The factors cleaning cycle duration, cleaning temperature and the detergent type used all have influence on the microbial reduction. With higher cleaning temperatures, the LR increases independent of the detergent type. Longer cleaning cycles and use of detergent increase the LR. Higher final rinsing temperatures give higher LRs.

**Conclusion:** The method is able to determine differences in the tested parameters duration, temperature and use of detergent. Both test strains show similar responses, so *M. luteus* can be used as test strain in future. The method can help in finding the necessary balance between energy saving and an acceptable level of hygiene so that no dangers arise by ever stricter eco-label regulations.

1. Nix ID, Frontzek A, Bockmühl DP (2015) Characterization of Microbial Communities in Household Washing Machines. *Tenside Surfactants Deterg* 52:432–440
2. Zalar P, Novak M, Hoog GSDE, Gunde-Cimerman N (2011) Dishwashers - A man-made ecological niche accommodating human opportunistic fungal pathogens. *Fungal Biol* 1–11
3. Brands B, Bockmühl DP (2015) Experimental Evaluation of Hygienic Conditions in Domestic Dishwashers. *Tenside Surfactants Deterg* 52:148–154

### 676-FMP

Antimicrobial effect of cold atmospheric plasma on stainless steel cutting tool surfaces

\*T. Albert<sup>1</sup>, A. J. Dittrich<sup>1</sup>, A. Lehmann<sup>1</sup>, T. Arnold<sup>1</sup>, P. G. Braun<sup>1</sup>

<sup>1</sup>University of Leipzig, Institute of Food Hygiene, Leipzig, Germany

Cutting tools can highly contribute to bacterial recontamination of food surfaces during slicing processes. The introduction of spoilage or pathogenic microorganisms can result in significant reduction of product shelf life or in potential health risks for consumer. Therefore, high hygienic standards are required including effective and sustainable decontamination technologies.

In this context the objective of this study\* was to investigate the suitability of cold atmospheric plasma jet (APJ) treatment for the decontamination of cutting tool surfaces.

For this purpose surfaces of laboratory-scaled stainless steel coupons (Ø 20 mm) were artificially contaminated with stationary phase cultures of *Listeria* (*L.*) *monocytogenes*, *Serratia* (*S.*) *liquefaciens* and *Lactobacillus* (*L.*) *sakei*. By this, three strains of each species were separately exposed to a microwave-excited APJ for up to three minutes. The inactivation rate was estimated depending on different contamination levels (3-4 log; 6-7 log units/surface) as well

as on two different plasma gas mixtures (feeding gas: argon or helium).

In summary, a significant reduction of bacterial counts could be achieved for all tested isolates, but differences in sensitivity were observed between the various bacteria and even between the different strains of each tested species. A more marked reduction was obvious when testing the lower contamination level and using helium in the gas mixture. By this, the mean reduction rates after three minutes of exposure achieved for *S. liquefaciens*, *L. monocytogenes* and *Lactobacillus sakei* were 2,9 log, 3,1 log and 1,7 log, respectively.

The results demonstrate the suitability of APJ treatment to significantly reduce various bacteria on cutting tool surfaces. In order to implement this technology in future food processing more research is needed to improve the inactivation efficiency.

\*This research project is supported by the German Ministry of Economics and Technology (via AiF) and the FEI (Research Association of the German Food Industry, Bonn). Project AiF 19256 BR.

---

#### 677-FMP

Addition of essential oil components in food to reduce food spoilage moulds and yeasts

\*L. Nißl<sup>1</sup>, F. Westhäuser<sup>1</sup>, M. Noll<sup>1</sup>

<sup>1</sup>Coburg University of Applied Sciences, Institute for Bioanalysis, Coburg, Germany

Food safety is important to reduce food spoilage microorganisms and foodborne pathogens. However, food safety is challenging as customers' demand for natural preservatives is increasing. Essential oils and its components are an alternative as antibacterial and antimycotic food additives. To apply essential oils in food, the minimal inhibitory concentrations (MIC) *in vitro* and in food matrices have to be analysed.

In this study, the effect of twelve different essential oil components against fourteen food spoilage moulds and yeasts was investigated. MIC of each component and strain was tested via microdilution method. Cinnamaldehyde (CA) had the lowest MIC for all tested strains. However, CA is organoleptic and was therefore combined with other essential oil components via checkerboard method. Overall, 27 out of 91 combinations showed a synergistic effect and both respective component concentrations can be reduced without changing MIC.

Essential oil components in food frequently reduce their antimycotic efficacy and impact the taste, smell and texture of food matrices. To test these effects of CA in ketchup, we tested each ingredient and its combinations on the MIC of *Saccharomyces cerevisiae*. Only the ingredients salt and vinegar showed a synergistic effect with CA. In addition, we analysed the effect of CA and combinations of ketchup components on *S. cerevisiae* by live-dead staining in flow cytometry. CA showed not only a fungistatic, but also a fungicide effect. Upcoming studies will investigate the antimycotic effect of CA in the whole ketchup via challenge tests.

---

#### 678-FMP

Usage of UV-C treatment for bacterial decontamination on eggshells via mercury vapor lamp and LED technology

\*S. Fleischmann<sup>1</sup>, S. Opherden<sup>1</sup>, T. Alter<sup>1</sup>, K. Rosen<sup>2</sup>, M. Bielicke<sup>2</sup>, I. Szabo<sup>3</sup>, S. Hadziabdic<sup>3</sup>, A. Gensch<sup>4</sup>, P. Rotsch<sup>5</sup>, G. Wiese<sup>6</sup>, U. Roesler<sup>2</sup>

<sup>1</sup>Freie Universität Berlin, Institute of Food Safety and Food Hygiene, Berlin, Germany

<sup>2</sup>Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany

<sup>3</sup>Federal Institute for Risk Assessment (BfR), NRL for the Analysis and Testing of Zoonoses (Salmonella), Berlin, Germany

<sup>4</sup>Löwendorfer Geflügelhof GmbH (LGH), Ahrensdorf, Germany

<sup>5</sup>OSA Opto Light GmbH, Berlin, Germany

<sup>6</sup>SKS Sondermaschinen- und Fördertechnikvertriebs- GmbH, Berlin, Germany

The bacterial contamination of eggshells with food-borne zoonotic agents in the commercial egg production is an important One Health issue. The traditional Ultraviolet-C (UV-C) mercury vapor lamp technology is already used for bacterial decontamination on eggshells in individual packaging lines in Germany. A novel system of UV-C light-emitting diodes (LEDs) poses an alternative providing many ecological and economic advantages.

The aim of the study was to develop and establish a UV-C LED light method for the commercial application in egg packaging lines. Therefore, the production parameters from an egg packaging line were determined. Thereafter, the bacterial decontamination efficiency of the UV-C LED light on ESBL-producing

*Escherichia (E.) coli*, *Enterococcus (E.) faecium*, *Campylobacter jejuni*, Methicillin-resistant *Staphylococcus aureus*, and *Salmonella* on the eggshell surface with and without organic load, was evaluated and compared to the traditional UV-C light method. For this purpose, the surfaces were inoculated with a defined bacterial concentration and the bacterial count was estimated before and after UV-C treatment.

Finally, a reproducible method for detecting the decontamination efficiency via UV-C on eggshells was developed and established. A high organic load of 10g bovine serum albumin (BSA) and 10g yeast extract resulted in a protective effect of Gram-negative bacteria against UV-C treatment of up to 2log CFU/cm<sup>2</sup> in contrast to no organic load. Gram-positive bacteria are less vulnerable to UV-C and stable in their detection rate with and without BSA. A high contamination dose of 10<sup>7</sup> CFU/cm<sup>2</sup> with *E. coli* showed only 1log CFU/cm<sup>2</sup> reduction after UV-C treatment. In contrast, under a contamination dose ≤ 10<sup>5</sup> CFU/cm<sup>2</sup> *E. coli* was no longer detectable. High contamination dose of *E. faecium* of 10<sup>7</sup> CFU/cm<sup>2</sup> showed only 0,5log reduction and 1log reduction using contamination doses ≤ 10<sup>5</sup> CFU/cm<sup>2</sup> after UV-C treatment. In general, field isolates from egg-producing poultry farms showed an increased UV-C tolerance. The comparability of the mercury vapor lamps and the LED panels is possible if the wavelength and the radiation intensity [W/m<sup>2</sup>] are similar.

---

#### 679-FMP

Impact of cold atmospheric plasma on inactivation of norovirus surrogates on stainless steel

\*T. Albert<sup>1</sup>, M. Reinhardt<sup>1</sup>, U. Truyen<sup>1</sup>, P. G. Braun<sup>1</sup>

<sup>1</sup>University of Leipzig, Institute of Food Hygiene, Leipzig, Germany

Infections with human noroviruses (NoV) are one of the most frequent reasons for gastroenteritis cases worldwide. NoV are highly contagious and spread through multiple routes of transmission including person-to-person and upon exposure to contaminated food, water or aerosolized vomitus particles. The viruses are extremely stable in the environment and resistant to many common disinfections and food processing

techniques. By this NoV can persist in different food as wells as on surfaces, which can be an important source of contamination.

For that reason the aim of the present study was to investigate the antiviral effect of cold atmospheric plasma jet (APJ) treatment on stainless steel surfaces. Until recently, no suitable cell-culture assay is available for the propagation of human norovirus particles and therefore suitable surrogate viruses are an option to be used in inactivation studies.

Laboratory-scaled stainless steel coupons (Ø 20 mm) were artificially contaminated with suspensions of two different norovirus surrogates (murine Norovirus MNV S99, Tulane Virus CCHMC ID: RT 0450). The dried virus suspensions were exposed to a microwave-excited spot-shaped APJ for up to three minutes. The inactivation was estimated depending on treatment time as well as on two different plasma gas mixtures (feeding gas: argon or helium). The detection of infectious particles of murine Norovirus as well as of Tulane virus was done by endpoint titration using two different cell lines (RAW 264.7 and LLC-MKS).

Results will be presented and discussed.

---

## 680-FMP

### Comparison of RAPD-PCR and MALDI-TOF MS for typing and identification of coliform bacteria

\*C. Reitter<sup>1</sup>, F. Schwab<sup>1</sup>, A. Tiehm<sup>1</sup>, J. Gescher<sup>2</sup>, M. Hügler<sup>1</sup>

<sup>1</sup>TZW: DVGW-Technologiezentrum Wasser, Microbiology and Molecular Biology, Karlsruhe, Germany

<sup>2</sup>Karlsruhe Institute of Technology, Applied Biology, Karlsruhe, Germany

**Introduction:** Coliform bacteria are indicators for the microbial drinking water quality. Identification of these indicator bacteria represents an important step during the search for a possible contamination source of drinking water. For this reason a fast and accurate method is a precondition for further decisions like e.g. boiling advices or disinfection procedures. However, precise identification of coliform bacteria is often very difficult as they are closely related. Particularly within the genus *Enterobacter* environmental and hygienic-relevant bacteria occur. In this case a combination of identification and strain typing could be used to discriminate closely related clinical from environmental strains.

**Objectives & Methods:** A commonly used typing method is the randomly amplified polymorphic DNA (RAPD), a genetic fingerprinting method. Much faster is the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS). It is applied to identify bacteria based on the protein composition within the cell. So far the method is mainly used for clinical bacteria, whereas its application for environmental strains is currently still limited. Therefore the aim of this study was to compare the RAPD with MALDI-TOF for typing and identification of coliform bacteria from (drinking) water samples with a focus on the genera *Enterobacter*, *Lelliottia*, *Serratia*, *Citrobacter* and *Buttiauxella*. This should answer the question whether these methods are sensitive and specific enough for strain comparison. The results were checked against identification results using multi locus sequence analysis (MLSA).

**Results & Conclusion:** It could be shown that typing of coliform bacteria is possible with both methods. Typing is also the first step in identifying missing strains in the database of MALDI-TOF MS, as was the case with *Lelliottia* species. Even if the database is not designed for

environmental bacteria and is therefore still incomplete, the method has great potential as a routine identification and typing method not only for the clinical but also for the drinking water and environmental sector.

---

## 681-FMP

Inhibition of menaquinone synthesis decreases resistance against freeze-thaw stress and growth rate in refrigerated milk of *Listeria monocytogenes*

\*A. Flegler<sup>1</sup>, V. Kombeitz<sup>1</sup>, A. Lipski<sup>1</sup>

<sup>1</sup>University of Bonn, Food Microbiology and Hygiene, Bonn, Germany

*Listeria monocytogenes* is one of the major food-related pathogens and causes listeriosis, a potentially life-threatening illness. One of the most challenging tasks during growth at low temperatures is maintaining cytoplasmic membrane fluidity by modification of lipid membrane composition. For *L. monocytogenes* the dominating adaptation effect is shortening of fatty acid chain length. However, for some strains an additional adaptive response is the increase of menaquinone concentration during growth at low temperatures. The increase of this neutral membrane lipid produced a fluidization of the membrane under low temperature conditions and therefore represents a fatty acid-independent adaptation mechanism. In this study menaquinone content for *L. monocytogenes* strains was reduced by supplementation with aromatic amino acids, which caused a feedback inhibition of the quinone synthesis pathway. Menaquinone-reduced grown *L. monocytogenes* strains and unaffected reference cultures were subjected to freeze-thaw cycles. Cryotolerance clearly decreased for cells with reduced menaquinone content. Growth experiments in milk at low temperature confirmed the growth-inhibiting effect of aromatic amino acids in *L. monocytogenes*. Reduced tolerance against freeze-thaw stress and decreased growth rates at low temperature conditions for menaquinone-depleted *L. monocytogenes* cultures confirmed the adaptive function of these neutral lipids for growth under low temperatures for *L. monocytogenes* and the potential of aromatic amino acids as protectants against this organism in food.

---

## 682-FMP

### Disinfection against ESBL producing *E. coli* on hatching broiler eggs – *in vitro* efficacy test and *in vivo* trials

G. Motola<sup>1</sup>, W. Tebrün<sup>2</sup>, M. Pees<sup>2</sup>, J. Bachmeier<sup>3</sup>, H. M. Hafez<sup>1</sup>, \*S.

Brüggemann-Schwarze<sup>1</sup>

<sup>1</sup>Institute for Poultry Diseases, Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

<sup>2</sup>Clinic for Birds and Reptiles, Veterinary Medicine, University of Leipzig, Leipzig, Germany

<sup>3</sup>Brütereisüd, BWE-Brütereisüd Weser-Ems GmbH & Co. KG, Regensburg, Germany

Bacterial contamination of poultry products with ESBL (extended-spectrum beta-lactamase) producing *E. coli* is of major concern for the public health sector, poultry industry and consumers. Hatching eggs can be considered carriers and potential contamination sources for one-day old broiler chicks, resulting in a higher risk of contaminated meat for the consumer. To prevent colonization with ESBL producing bacteria early in the broiler production chain, formaldehyde and hydrogen peroxide are used in commercial hatcheries to disinfect hatching eggs. This study focusses on testing the efficacy of six different disinfection methods against ESBL producing bacteria in broiler hatching eggs.



Using an artificial contamination with a CTX-M-1 producing *E. coli* (phylogroup B1) on eggshell samples, we first evaluated whether conventional and alternative methods can achieve an efficacious disinfection. In the next step, we assessed the effect on hatching rate and chicken development. In a subsequent trial under field conditions, we investigated 1) the disinfection efficacy with a large sample size, 2) the health status of the chicks after hatch and the 3) weight gain of broilers.

The six tested methods, namely formalin gassing, hydrogen peroxide + alcohol, essential oils applied as spray and cold fog, peracetic acid and low energy electron beam reduced the artificial ESBL contamination on the egg shell samples. Five of six methods had no negative effect on hatchability. Essential oils as spray reduced significantly the hatchability and was not included in field trials. In field trials, three out of four tested disinfected methods reduced the natural contamination level on hatching eggs and did not have negative effects on hatching rate nor chicken development.

---

### 683-FMP

#### Developments in the Biopesticide Industry and Implications for Food Safety Testing

\*D. Zommick<sup>1</sup>

<sup>1</sup>Valent BioSciences LLC, Biorational Crop Protection, Libertyville, United States

##### 1. Introduction

Trends in agriculture and environmental regulation (e.g. the Sustainable Use Directive from the European Commission (Art. 14, 2009/128/EC)) are favoring biopesticide adoption over conventional, synthetic plant protection products for crop protection. This sentiment is also reflected in consumer demand for fewer chemical residues and sustainably sourced food. Microbial biopesticides make up a diverse, growing subset of plant protection products which may impact the food safety industry as has been seen with *Bacillus thuringiensis*-based products.

##### 2. Objective

To highlight the potential for new microbial pesticides to appear in food safety screens current and projected trends in the biological crop protection industry are reviewed. Changes in agricultural practices that promote the use of biopesticides is also addressed.

##### 3. Methods

A review of market research reports, academic and corporate literature, and the European Commission Pesticide Database and pending Active Substance Authorizations was conducted with emphasis on microbial plant protection products which may appear in food safety screens.

##### 4. Results

The European biopesticide market is projected to increase in value at a rate of 15.6% over the next five years to represent 33% of the global biopesticide market. Over 40% of this value is from the sale of microbial products which are commonly applied to foliage. This trend is encouraged by dramatic regulator changes, such as the Ecophyto program in France, that have forced producers to find alternate methods of plant protection. Dozens of fungi, bacteria, yeasts, viruses and biochemicals are currently registered or

under review by the EU Commission and EFSA to meet this need.

## 5. Conclusions

As food producers in Europe identify methods to diminish reliance on synthetic pesticides and enhance Integrated Pest Management strategies, biopesticides will represent a larger share of crop protection tools and become more prevalent in food. There is a need for a concerted effort to understand the implications of biopesticides in food for the food safety industry with clear guidance on how registered microbes can be monitored differently than native strains.

---

### 684-FMP

#### Quantification of *Pseudomonas* in raw milk and correlation with farm-specific influencing factors

\*K. Hofmann<sup>1</sup>, A. Siebert<sup>1</sup>, M. Wenning<sup>2</sup>, S. Scherer<sup>1</sup>

<sup>1</sup>Technische Universität München, Lehrstuhl für Mikrobielle Ökologie, Freising, Germany

<sup>2</sup>Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany

*Pseudomonas* is a frequent genus of the microbiota of raw milk. Due to its psychrotrophic properties, it is able to grow and produce exoenzymes during cold storage until processing of the milk in the dairies. These proteolytic and lipolytic enzymes are able to cause deterioration of end products, as they withstand ultrahigh temperature treatment. Critical factors for the spoilage potential are besides the duration and temperature of raw milk storage, the initial load with *Pseudomonas* on the farms.

Aim of this study was the determination of *Pseudomonas* counts in raw milk samples from various farms all over Germany in order to correlate high loads with farm specific factors like the type of milking system or animal husbandry. Therefore, the protocol for bacterial DNA extraction out of raw milk samples was optimized by using different EDTA concentrations for a better removal of fat and proteins. Besides this, preservation agents were tested in order to prevent bacterial growth during transportation of the samples. Moreover, *Pseudomonas* counts were determined by a species-specific qPCR assay as well as by high throughput amplicon sequencing.

Quantification of *Pseudomonas* by qPCR showed a good correlation to cell counts determined by either classical cultivation as well as by calculation based on sequencing data. The majority of tested milk samples showed low *Pseudomonas* loads with cell counts < 1.0E+03 cfu/ml. However, 31 out of 317 samples had values between 1.0E+04 cfu/ml and 1.0E+08 cfu/ml. The microbiome data obtained by amplicon sequencing of 184 samples showed an average genus diversity of 220 genera per sample. 36 samples had a relative amount of *Pseudomonas* above 1.5 %, with values up to 95 %.

Further investigations on contamination routes at the farms by site inspections and sampling as well as analyses of the effect of milk storage time are planned. By this, means to lower the *Pseudomonas* counts in raw milk shall be developed, which lead to a reduction of the enzyme load and subsequently to milk products of higher quality.

---

### 685-FMP

## Comprehensive analysis of raw milk microbiota from farms throughout Germany

\*A. Siebert<sup>1</sup>, K. Hofmann<sup>1</sup>, M. Wenning<sup>2</sup>, S. Scherer<sup>1</sup>

<sup>1</sup>Technical University of Munich, Chair of Microbial Ecology, ZIEL - Institute for Food and Health, Freising, Germany

<sup>2</sup>Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

Raw milk microbiota has a strong impact on the hygienic and sensory quality of the resulting end products. Profound knowledge of farm specific factors influencing the bacterial community composition of bulk tank milk is needed to strengthen positive effects and reduce negative consequences. Since Next-Generation sequencing facilitates the detailed investigation of complex microbial communities, the analysis of the raw milk microbiome gained increasing attention. However, due to low cell counts and a highly complex matrix, it is difficult to prepare bacterial DNA from raw milk for sequencing.

Therefore, in this study, an optimized method for sequencing library preparation was established. For this, different cycle numbers were tested to investigate whether and to which extend PCR introduces artifacts into sequencing data. In order to assess influencing factors on the raw milk biodiversity with regard to spoilage associated bacteria and indicators for hygiene, farms were selected from all over Germany. Logistics for raw milk sampling have been set up with a focus on geographical position, type of production, and size of the herd as these parameters may determine the biodiversity of the respective raw milk. Finally, the microbiota of raw milk samples is evaluated by high-throughput amplicon sequencing using an Illumina platform and their diversity is analyzed with IMNGS and Rhea.

For sample preparation, it was shown that PCR cycle number during sequencing library preparation had a significant impact on the determination of the raw milk microbiota. This demonstrates the relevance of PCR conditions during library preparation, as those may influence the estimation of the abundance of particular bacterial groups. First results from milk samples will be presented and analyzed regarding a possible correlation between microbiota and farm specific factors. Eventually, through the evaluation of farm specific influencing factors, an improvement in microbial quality of German raw milk should be achieved.

### 686-FMP

Investigations on the occurrence of *Cryptosporidium* spp. in game and raw consumed food in Germany – Is there a potential human health relevance?

\*C. Jäckel<sup>1</sup>, K. Nöckler<sup>1</sup>, M. Richter<sup>1</sup>, J. A. Hammerl<sup>1</sup>, A. Mayer-Scholl<sup>1</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany

**Questions:** Cryptosporidiosis is a widespread diarrheal disease of humans and animals, caused by the protozoan parasite *Cryptosporidium*. The pathogen is mainly ingested by the consumption of water or raw food, contaminated by the contact with faeces of infected persons or animals. However, there is only insufficient information on the worldwide distribution of *Cryptosporidium*. For a better understanding of the risk of human cryptosporidiosis by consumption of contaminated food in Germany, this study investigated the occurrence in game and food products with low processing degree (i.e. salads, herbs).

**Methods:** In this study 229 wildlife samples from eight districts in Brandenburg (hunting season 2017/18), as well as

194 salads/herbs were tested for the presence of *Cryptosporidium*. Extracted samples were molecularly checked for the presence of three gene sequences (18S rDNA, GP60, COWP) using nested PCR and specified by sequencing and RFLP. Additionally, salads and herbs were also examined by fluorescence microscopy.

**Results:** First results indicate that the molecular prevalence of *Cryptosporidium* in game of Brandenburg is high (wild boar: 24.11%, red deer: 10.53%, roe deer: 42.37%), whereas young animals seem to be more affected than older. Additionally to the wild boar and deer specific species *C. scrofarum*, *C. suis*, and *C. sp.* deer specific genotype, human pathogenic species *C. parvum* and *C. ubiquitum* were also identified. In contrast to game, *Cryptosporidium* could neither be molecularly nor microscopically detected in salads/herbs.

**Conclusion:** The pathogen seems to be autochthonous in German game but not detectable in salads and herbs available in Germany. For the assessment of the potential risk which may arise from the consumption of such food a monitoring of raw consumed food and game should be established.

### 687-FMP

Antibiotic resistance in *Escherichia coli* from broilers after amoxicillin treatment

\*E. Burow<sup>1</sup>, M. Grobbel<sup>1</sup>, B. A. Tenhagen<sup>1</sup>, S. Banneke<sup>2</sup>, A. Käsbohrer<sup>1,3</sup>

<sup>1</sup>German Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany

<sup>2</sup>German Federal Institute for Risk Assessment, Department Experimental Toxicology and ZEBET, Berlin, Germany

<sup>3</sup>University of Veterinary Medicine, Institute for Veterinary Public Health, Berlin, Germany

**Questions:** Resistance rates to penicillin are high in commensal *Escherichia (E.) coli* from German monitored chickens. It was questioned whether administration system impacts occurrence of antibiotic resistance in a group of treated and untreated chickens. Hence, we aimed to investigate resistance to antibiotics in *E. coli* from chickens" cloak after oral individual (I) or group treatment (G) with amoxicillin, or after contact to I or G (KI or KG) compared to chickens untreated (C) or pre-treatment.

**Methods:** I/KI, G/KG and C (23 chickens per group) were housed in three separate rooms. The study started when the chickens were two days old. Chickens in I and G were treated with amoxicillin for five days at the beginning of the study period. In a second treatment period, G, KG and C received amoxicillin from day 34 to 38. Cloacal swabs were taken repeatedly (at study days 0, 2 to 7, 14, 21, 28, 34 to 38).

**Results:** Initial resistance rates were high in the treated and untreated groups. Resistance to ampicillin and further antibiotic agents increased significantly in G and I, and on a significantly lower level in KI, KG and C during the first treatment period. After the treatment, resistance rates declined to initial or lower values in all groups. During a second treatment, resistance levels were equally high in all treated groups G, KG and C. Cephalosporin resistance was generally low and did not change over the experimental period.

**Conclusions:** Initial resistance levels were generally high but still, antibiotic treatment increased antibiotic resistance within two days. Co-selection of resistance to several antibiotic classes was indicated. Repeated treatment was

followed by comparable high resistance levels as during the first treatment or once-treated-animals.

---

## 688-IIP

### The Cyclophilin PpiB Connects Cysteine Metabolism with Regulation of Toxin Production in *Clostridium difficile*

\*M. S. Karagöz<sup>1</sup>, C. M. Ünal<sup>1,2</sup>, M. Berges<sup>1,3</sup>, C. Priebe<sup>1</sup>, J. M. Borrero de Acuna<sup>1</sup>, J. Wissing<sup>3,4</sup>, L. Jänsch<sup>3,4</sup>, D. Jahn<sup>1,3</sup>, M. Steinert<sup>1,3,5</sup>

<sup>1</sup>TU Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany

<sup>2</sup>Turkish German University, Molecular Biotechnology, Istanbul, Turkey

<sup>3</sup>Braunschweig Integrated Centre of Systems Biology, Braunschweig, Germany

<sup>4</sup>Helmholtz Centre for Infection Research, Cellular Proteomics Research, Braunschweig, Germany

<sup>5</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

*Clostridium (Clostridioides) difficile* is an anaerobic, toxin producing, enteric pathogen that causes typically antibiotic-associated hospital acquired infections ranging from mild diarrhea to toxic megacolon with high recurrence rates and fatal outcome. Cyclophilins belong to the family of peptidyl-prolyl-cis/trans-isomerases (PPlases), which are ubiquitous proteins with protein folding and chaperoning activities. As such they participate in general protein maturation pathways, as well as, in specific protein-protein interactions supporting unique phenotypes related to important physiological or pathogenic features. While in Gram-positive and Gram-negative pathogens, including *Listeria monocytogenes* and *Legionella pneumophila*, contribution of PPlases to various virulence mechanisms have been shown, very little is known about these proteins of *C. difficile*. Thus, we analysed the only cyclophilin of *C. difficile*, PpiB, using an interactomic approach. Here, we present a distinct subset of PpiB interaction partners, which majorly belong to the central energy and amino acid metabolism, and stress response. Also, several members of regulation of transcription/translation, protein folding, transport and motility are associated with PpiB. Within the central metabolism CysK, the terminal enzyme in cysteine biosynthesis, stands out, where a functional interaction between PpiB and CysK was shown. This explains the increased cysteine-susceptibility and deregulation of cysteine-dependent toxin production in the *ppiB*-deletion mutant and highlights the contribution of PpiB to the pathogenicity of *C. difficile*.

---

## 689-IIP

### Deciphering the cross-talk between host and pathogen during intracellular persistence of *Staphylococcus aureus* within mast cells

\*T. Sauerwein<sup>1</sup>, O. Goldmann<sup>2</sup>, E. Medina<sup>2</sup>, K. Förstner<sup>1</sup>

<sup>1</sup>ZB MED, Köln, Germany

<sup>2</sup>Helmholtz Centre for Infection Research, Braunschweig, Germany

## 1. Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease where patients suffer from skin lesions that in 75 % up to 100 % of the cases are colonized by *S. aureus*. *S. aureus* can induce degranulation of mast cells which accumulate in AD lesions and thereby exacerbating the inflammation. During degranulation, mast cells release antimicrobial peptides and proteases with bactericidal effect. However, *S. aureus* can evade these extracellular antimicrobial mechanisms of mast cells by internalizing and persisting within mast cells. As the number of mast cells is increased in AD lesions and *S.*

*aureus* persists within these long-life cells, it is likely that the *S. aureus* within mast cells contributes to chronic carriage in AD patients. In order to survive within mast cells, *S. aureus* undergoes extensive gene expression reprogramming including genes involved in cell wall biogenesis that results in increased cell wall thickness. Furthermore, a recent study reported that *S. aureus* manipulates mast cell gene expression in order to facilitate its own uptake by these cells.

## 2. Objectives

In this study, a dual RNA-seq approach, which enables the parallel assessment of the transcriptional response of both interaction partners, was used to get further insights into the mechanisms underlying the *S. aureus* survival strategies and to identify the pathways in mast cells manipulated by *S. aureus*.

## 3. Material and Methods

Dual RNA-sequencing and bioinformatical analysis.

## 4. Results

The analysis yielded a large number of up- or down regulated genes in host and pathogen cells. To discover possible master regulators, gene sets discovered in a gene set enrichment analysis are being compared to gene sets that have a predicted interaction with a (predicted or validated) sRNA. Additionally the RNA-seq data is being analyzed using ANNOgesic to annotate so far unknown sRNAs.

## 5. Conclusion (preliminary)

Dual-RNA-seq of *S. aureus* and human mast cells uncovers a large number of up- or down regulated genes during infection.

---

## 691-IIP

Epigenetic mechanisms regulate host innate immunity against uropathogenic and commensal-like *Escherichia coli*

\*K. Mukherjee<sup>1</sup>, U. Dobrindt<sup>1</sup>

<sup>1</sup>University of Münster, Institute of Hygiene, Münster, Germany

## Introduction

Innate-immunity-related genes in humans are activated during urinary tract infections (UTIs) caused by pathogenic strains of *Escherichia coli* but are suppressed by commensals. Epigenetic mechanisms play a pivotal role in the regulation of gene expression in response to environmental stimuli.

## Objectives

To determine the role of epigenetic mechanisms such as DNA methylation, histone acetylation and miRNAs in regulating the different behaviors of uropathogenic and commensal-like *E. coli* strains.

## Materials & methods

We infected larvae of the greater wax moth, *Galleria mellonella*, a widely used model insect host, with uropathogenic *E. coli* (UPEC) strain CFT073 that causes symptomatic UTIs in humans or a commensal-like strain

83972 that causes asymptomatic bacteriuria (ABU). DNA, RNA and histone proteins were isolated from infected larvae for the detection of DNA methylation, histone acetylation, and miRNA expression by next-generation sequencing, RTPCR, and ELISA.

## Results

Infection with the UPEC strain was more lethal to larvae than infection with the attenuated ABU strain due to the recognition of each strain by different Toll-like receptors, ultimately leading to differential DNA methylation, histone acetylation and miRNA expression. We correlated epigenetic changes with the induction of innate-immunity-related genes. Transcriptomic analysis of infected *G. mellonella* larvae infected with *E. coli* strains CFT073 and 83972 revealed strain-specific variations in the class and expression levels of genes encoding antimicrobial peptides, cytokines, and enzymes controlling major epigenetic mechanisms.

## Conclusions

Our results provide evidence for the differential epigenetic regulation of transcriptional reprogramming by UPEC and ABU strains of *E. coli* in *G. mellonella* larvae, which may be relevant to understanding the different behaviors of these bacterial strains in the human urinary tract.

---

## 692-IIP

### Host Innate Immune Reaction to Erythrocytes Lysed by the Malaria Parasite *Plasmodium Falciparum*

\*A. González Delgado<sup>1</sup>, S. Imscher<sup>1</sup>, T. Reiß<sup>2</sup>, G. Pradel<sup>2</sup>, C. Skerka<sup>1</sup>

<sup>1</sup>Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

<sup>2</sup>RWTH Aachen University, Aachen, Germany

Malaria tropica is an infectious disease caused by the unicellular parasite *Plasmodium falciparum*. This parasite invades and replicates within the red blood cells (RBCs) causing the destruction of these cells.

Factor H related protein 1 (FHR1) is a negative regulator of the terminal alternative complement pathway and competes with factor H in binding to C3b. Recently our group identified a new function of FHR1.

FHR1 binds to necrotic cell surfaces and mediates inflammation through the activation of the NLRP3 inflammasome in monocytes. FHR1 distinguishes necrotic from apoptotic surfaces and therefore represents an important mediator of inflammation of necrotic surfaces. Thus, we asked whether destruction of RBCs by *P. falciparum* is recognized by FHR1 thereby triggering strong inflammation and enhancing malaria pathology.

In vitro experiments show that heat-induced necrotic RBCs bind FHR1, which in turn triggers the production of pro-inflammatory cytokines.

Furthermore, necrotic RBCs bind FHR1 via the N-terminus presenting the C-terminus to immune cells. These binding characteristics are confirmed on necrotic malaria-induced RBCs. We hypothesize that malaria patients expressing FHR1 develop a more severe pathology compare to those that are FHR1 deficient. This could explain why about one third of the Nigerian population presents with a FHR1 deficiency. Whether the FHR1 plasma concentration increases in malaria patients, needs further evaluation.

## 693-IIP

Domain composition and variations of immune evasion protein C (PspC) among ***Streptococcus pneumoniae*** strains.

\*S. Du<sup>1</sup>, C. Vilhena<sup>1</sup>, S. King<sup>2</sup>, M. Rohde<sup>3</sup>, C. Skerka<sup>1</sup>, S. Hammerschmidt<sup>4</sup>, P. F. Zipfel<sup>1</sup>

<sup>1</sup>Leibniz insititue for natural product research and infection biology, Infection biology, Jena, Germany

<sup>2</sup>Nationwide Children's Hospital, Center for Microbial Pathogenesis, Columbus, Ohio, Germany

<sup>3</sup>Helmholz Center for Infection Research, Central Facility for Microscopy, Braunschweig, Germany

<sup>4</sup>Interfaculty Institute for Genetics and Functional Genomics, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

*S. pneumoniae* is an important human gram positive pathogenic bacterium that can cause infections including otitis media, even acute life-threatening infections like meningitis and sepsis. In order to evade host complement attack, pneumococci have evolved a number of mechanisms. The surface protein PspCs represent important pneumococcal adhesive and immune evasion proteins and promising vaccine candidates. PspC binds several human plasma proteins like factor H, plasminogen, C3, C4BP, secretory IgA, vitronectin and thrombospondin-1. PspC combines antigenic diversity with common binding patterns. At present the NCBI data bank includes 53955 records of PspC variants (Oct. 2019). In order to explain the variability of these many PspC entries, by in silico analysis, sequence comparison and alignment we define a clear domain architecture of the variants. The domains include (0) a signal peptide, (1) hypervariable domain (HVD), (2) repeat domains, (3) random coil domain, (4) a proline rich region (PRD) and (5) a surface anchor, of either a modular choline binding domain (CBD) or an anchor with an LPsTG motif.

This approach identified the five known domains but also seven new domains, and furthermore new domain subtypes, some of which are unique to one variant. According to the homology of full length PspC proteins, they can be grouped into two classes with a CBD or LPsTG motif respectively. The composition, usage, and homology of individual domains differ among the selected variants. Each domain has specific features. And the HVD shows the highest degree of sequence variations. In addition a structural analysis revealed differences in the composition of the outside oriented N-terminal region and of the C-terminal region which includes capsule spanning and membrane anchoring domains. The N-terminal region is rich in charged residues and mainly composed of  $\alpha$ -helices. In contrast, C-terminal domains have less charged residues. This sequence-based in silico approach allows a better structure comparison of the variants, is also helpful to vaccine approaches and to define the domain composition of new PspC variants in particular isolated from clinical strains.

---

## 694-IIP

Effects of stress on 2-O methylation and immune stimulation of tRNA in ***E. coli***

\*A. Grober<sup>1</sup>, I. Freund<sup>2</sup>, A. Galvanin<sup>3,4</sup>, V. Marchand<sup>4</sup>, Y. Motorin<sup>3,4</sup>, A. Dalpke<sup>1</sup>

<sup>1</sup>TU Dresden, Institute of Medical Microbiology and Hygiene, Dresden, Germany

<sup>2</sup>Ruprecht-Karls University Heidelberg, Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg, Germany

<sup>3</sup>BioPole Lorraine University, IMoPA UMR7365 CNRS-UL, Vandoeuvre-les-Nancy, France

<sup>4</sup>BioPole Lorraine University, Next-Generation Sequencing Core Facility, Vandoeuvre-les-Nancy, France

Posttranscriptional RNA modifications play a recognized role in self/non-self discrimination of nucleic acids in innate immunity by endosomal Toll-like receptors TLR3, 7 and 8. Previous work has identified a naturally occurring 2"-O-methylation of guanosine at position 18 (Gm18) within tRNAs, which acts as a suppressive ligand for TLR stimulation within a defined sequence: Transfection of PBMCs with modified RNA (Gm18) together with otherwise stimulatory RNA inhibited the immune response.

## 2. Objectives

We previously observed that the Gm level globally increased in the lab strain *E. coli* DH5 $\alpha$  under stress conditions. As Gm18 methylations led to reduced stimulatory activity in earlier studies, we wanted to investigate whether stress conditions have an impact on the methylation pattern and immune-stimulation of RNA from *E. coli* isolates.

## 3. Materials & methods

Clinical *E. coli* strains were cultured under normal growth conditions or stress conditions induced by either nutrient deprivation or antibiotics. Bacterial tRNA and rRNA were purified separately from total RNA, then human peripheral blood mononuclear cells (PBMCs) were transfected with these RNAs and IFN $\alpha$  and TNF $\alpha$  production were quantified in the supernatants. By using the data from ELISA and flow cytometry, we determined how the modified RNA from *E. coli* contributes to stimulatory or suppressive immune response. Methylation patterns were determined by RiboMethSeq method.

## 4. Results

The cultivation of clinical *E. coli* strains under stress conditions (antibiotics) compared to unstressed conditions led to a significantly lower immune stimulation in tRNA and gRNA.

## 5. Conclusion

The methylation level of tRNA has been demonstrated as an immunosuppressive regulator compared to total or rRNA from bacteria. Here we show that tRNA isolated from stressed *E. coli* is more immunosuppressive than tRNA isolated from unstressed *E. coli*. These interesting findings raise the link how stress-induced methylation of RNA supports the immune-escape strategy of *E. coli* in physiological or pathological conditions.

---

### 695-IIP

#### Impact of H1N1 virus and *Streptococcus pyogenes*

coinfection on coagulation and microbiome in mice  
J. Köhler<sup>1,2</sup>, K. Study Group<sup>1,2</sup>, B. Kreikemeyer<sup>1,2</sup>, \*S. Oehmcke-Hecht<sup>1,2</sup>

<sup>1</sup>Rostock University Medical Center, Institute of Medical Microbiology, Virology and Hygiene, Rostock, Germany

<sup>2</sup>University Medicine Rostock, Rostock, Germany

#### Impact of H1N1 virus and *Streptococcus pyogenes* coinfection on coagulation and microbiome in mice

Juliane Köhler, KolInfekt Study Group, Bernd Kreikemeyer and Sonja Oehmcke-Hecht

### Introduction

The upper respiratory tract is a microbial niche of a diverse bacterial community and a portal of entry for many potential pathogens, including *Streptococcus pyogenes*, a strictly human pathogen. Seasonal influenza infections can lead to an increased risk of dissemination of bacteria to the lower respiratory tract or to secondary bacterial infections, like pneumonia.

### Objectives

To gain a better understanding of the co-pathogenesis of influenza viruses with *S. pyogenes* in the lung.

### Material and Methods

We established a sequential intranasal coinfection model in C57BL/6J mice. On day 0 IAV H1N1 were applied intranasally and 2 days after, mice were inoculated intranasally with *S. pyogenes*. 9 days post infection we analyzed clotting times, survival rates, bacterial and viral dissemination in kidney, blood and lung, as well as tissue pathology. Moreover, the impact of mono- und co-infection on the murine microbiome of the respiratory and intestinal tract was investigated.

### Results and Conclusion

Intranasal mono infection with *S. pyogenes* or Influenza A H1N1 showed no clinical symptoms in mice. After sequential co-infection we detected an increase of neutrophils in the blood. The aPTT clotting time was significantly prolonged and septic mice were positive for the H1N1 virus in the lung. Mortality rate was 45% after coinfection. Analysis of the gut microbiome showed systemic effects on the intestinal microbiota in infected mice, e.g. increased Akkermansiaceae. Additionally, Ruminococcaceae were increased in septic co-infected mice.

---

### 696-IIP

withdrawn

---

### 697-IIP

Immunization with TLR2-triggering lipidated pneumococcal antigens enhances and skews antibody responses and leads to reduced colonization

\*F. Voß<sup>1</sup>, L. F. van Beek<sup>2,3</sup>, D. Schwudke<sup>4</sup>, T. H. A. Ederveen<sup>5</sup>, F. J. van Opzeeland<sup>2,3</sup>, D. Thalheim<sup>1</sup>, S. Werner<sup>1</sup>, M. I. de Jonge<sup>2,3</sup>, S. Hammerschmidt<sup>1</sup>

<sup>1</sup>Interfaculty Institute for Genetics and Functional Genomics, Center for Functional Genomics of Microbes, University of Greifswald, Department of Molecular Genetics and Infection Biology, Greifswald, Germany

<sup>2</sup>Radboud Institute for Molecular Life Sciences, Radboudumc, Section Pediatric Infectious Diseases, Laboratory of Medical Immunology, Nijmegen, Netherlands

<sup>3</sup>Radboudumc, Radboud Center for Infectious Diseases, Nijmegen, Germany

<sup>4</sup>Research Center Borstel, Leibniz Center for Medicine and Bioscience, Priority Area Infection, Division of Bioanalytical Chemistry, Borstel, Germany

<sup>5</sup>Center for Molecular and Biomolecular Informatics, Radboud University Medical Center, Radboud Institute for Molecular Life Sciences, Nijmegen, Netherlands

**Introduction** Pneumococcal vaccines have important limitations, including restricted serotype coverage facilitating replacement by non-vaccine serotypes and high manufacturing costs. Therefore, one direction of research is engaging in the development of a serotype-independent and protein-based vaccine.

**Objectives** Recently, we have shown that immunization with the non-lipidated pneumococcal lipoproteins DacB and PnrA leads to reduced pneumococcal colonization. In this study, we have investigated the effects of lipidation and vaccination route (intranasal or subcutaneous) on the level of protection and humoral as well as cellular immune responses.

**Materials & methods** In a mouse model of colonization, we compared the immunogenicity and protectivity of lipidated versus non-lipidated DacB or PnrA with and without additional adjuvant depending on the immunization route. Antigen-specific systemic IgG and IgG subclass levels in antisera were determined by ELISA. Cytokine profiles after intranasal pneumococcal challenge in the NALT (local) and in supernatants after spleen cell stimulation (systemic) were analyzed by flow cytometry. Lipidated proteins triggering the TLR2 were shown by HEK-Blue™ hTLR2 cell reporter assays.

**Results** Immunization of mice with TLR2-engaging lipidated proteins induced increased IgG levels independent of the application of additional adjuvant. Humoral immune responses were characterized by a Th1-skewed IgG phenotype indicated by elevated levels of IgG2 compared to non-lipidated proteins inducing a IgG1-dominated profile. In addition, mice immunized with the lipidated proteins showed reduced bacterial loads in the nasal tissue compared to the non-lipidated proteins. However, no clear cytokine profile associated with protection of lipidated proteins could be determined.

**Conclusion** In principle, lipoproteins are interesting candidates for future vaccine strategies as they are conserved, abundant, and immunogenic. We showed the potential of lipidated proteins DacB and PnrA to induce protection against pneumococcal colonization. Therefore, protein lipidation might represent an attractive approach for the development of novel pneumococcal vaccines.

*Dermabacter hominis*, *Micrococcus luteus*, and *Rothia* sp. were isolated from sputum of CF patients. To monitor pathogen-commensal interaction, the strains were co-cultured with a mCherry-expressing *P. aeruginosa* PA01 strain at 370C in Columbia broth. Moreover, to study the host impact, airway epithelial cells (Beas2B) were co-cultured with a commensal strain and PA01, and incubated overnight. Supernatants were tested for IL-8 secretion.

## Results

Results from a previous microbiome study of 106 CF patients showed that *P. aeruginosa* was positively correlated with levels of IL8, TNF $\alpha$  and IL-1 $\beta$  in the sputum. Inversely, richer microbiomes characterized by high abundance of commensal bacteria were linked to lower levels of inflammation. Experiments now show that members of *Streptococcus* spp. possess potent inhibitory activity against PA01, especially *S. cristatus*, *S. mitis*, and *S. oralis*. Other potential members include *S. gordinii*, *S. sanguinis*, *S. parasanguinis*, and *S. salivarius*. On the other hand, commensals belonging to other genera did not show growth-inhibitory activities against PA01. Co-cultures of Beas2B cells with members of *Streptococcus* sp. lowered IL8 secretions compared to monocultures with PA01 alone.

## Conclusion

Commensal bacteria, especially members of the *Streptococcus* sp., may provide protective effects against *P. aeruginosa* infection by inhibiting its growth and reducing inflammation.

## 698-IIP

Commensal *Streptococcus* spp. may possess protective effects against *Pseudomonas aeruginosa* in airway epithelial cells in the context of cystic fibrosis

\*A. Tony-Odigie<sup>1</sup>, S. Boutin<sup>1,2</sup>, A. Dalpke<sup>1</sup>

<sup>1</sup>Institute of Medical Microbiology and Hygiene, Dresden, Germany

<sup>2</sup>Translational Lung Research Center, DZL, Heidelberg, Germany

## Introduction

Microbiome studies in cystic fibrosis (CF) patients have shown relationships between microbiome, lung function and inflammation. It has been reported that microbiomes dominated by commensal bacteria of the oropharynx correlate with improved clinical conditions raising the hypothesis that commensal bacteria exert protective effects. Yet, the impact of airway commensals on immune response and infection remains controversial.

## Objectives

The current study aims to evaluate possible inhibitory and immunomodulatory effects of commensal bacteria isolated from CF patients against *P. aeruginosa* PA01 infection on airway epithelial cells.

## Materials and methods

84 aerobic commensals including members of *Streptococcus* sp., *Neisseria* sp., *Actinomyces oris*, *Corynebacterium* sp.,

## 699-IIP

### Applying bacteria-derived nucleotides as adjuvant in primary human cells

\*L. S. Chen<sup>1</sup>, A. Grober<sup>1</sup>, S. Leccese<sup>1</sup>, A. Dalpke<sup>1</sup>

<sup>1</sup>TU Dresden, Institute of Medical Microbiology and Hygiene, Dresden, Germany

## 1. Introduction

Sensing bacteria-derived nucleotides, e.g. bacterial RNA (bRNA) or second messenger cyclic dinucleotides (CDNs), by the innate immune system has been investigated intensively the last two decades. Accumulating data indicate bRNA and CDNs, recognized by endosomal TLRs and stimulator of interferon genes (STING), not only regulate bacteria physiology but affect host innate and adaptive immunity during infection, suggesting the potential application on vaccine development.

## 2. Objectives

Investigate adjuvant effect and signaling of bacterial nucleotides in host cells.

## 3. Materials & methods

Primary human peripheral blood mononuclear cells (hPBMC) were isolated from buffy coats and stimulated with either total bRNA from *Pseudomonas aeruginosa*, or CDNs bis-(3'-5')-cyclic dimeric guanosine monophosphate (GG) or cyclic [G(3",5")pA(3",5")p] (cGAMP). Supernatants were subjected to enzyme-linked immunosorbent assay (ELISA) for cytokine detection, and cells were analyzed for surface costimulatory molecules. For antigen (Ag)-specific recall response, CMV/pp65 peptide was used as stimulus, treated alone or in

combination with nucleotides. IFN $\gamma$ -secretion and Ag-specific proliferation of T cells were determined by flow cytometry.

#### 4. Results

bRNA-treated hPBMC secreted potent levels of proinflammatory cytokines, but subtle level of IFN $\alpha$ . In contrast, stimulation by GG selectively produced IFN $\alpha$  but not the others. Interestingly, adding bRNA into CDNs-treated cells increased the secretion of proinflammatory cytokines, but did not affect the level of costimulatory molecules, suggesting that activation of both pathways may provide potential adjuvant effects with less cytotoxic cytokines. To address this hypothesis, CMVpp65-mediated T cell recall responses were tested. hPBMC generated pronounced amounts of IFN $\gamma$ -producing T cells after 3 hours, comparing with CMVpp65 alone. Furthermore, bRNA plus GG promoted Ag-specific T cell proliferation after 7 days, supporting the adjuvant potential of intracellular nucleotides.

#### 5. Conclusion

Activation of either intracellular TLR or STING pathways could be applied as adjuvant.

#### 700-IIP

Shiga Toxin 2a Binds to Complement Components C3 and C5 and Upregulates their Gene Expression in Human Cell Lines

\*S. Kellnerová<sup>1</sup>, S. Chatterjee<sup>1</sup>, R. Würzner<sup>1</sup>, D. Orth-Höller<sup>1</sup>

<sup>1</sup>Medical University Innsbruck- Austria, Institute for Hygiene and Medical Microbiology, Innsbruck, Austria

Introduction: Shiga toxin 2a (Stx2a) is one of the main virulence factors involved in the development of Enterohemorrhagic *Escherichia coli* (EHEC) associated hemolytic uremic syndrome (eHUS). Its ability to activate complement via the alternative pathway and to interact with some of complement proteins is believed to be crucial for the progression of the disease. Although substantial advancements have been achieved in decoding the disease development, the role of complement in both gut and blood has only recently begun to be explored.

Objectives: The aim of this study is to investigate the interaction of Stx2a with the pivotal complement proteins C3 and C5 and to check whether Stx2a stimulation influences their transcription and synthesis in various human cell lines.

Methods: Enzyme-linked immunosorbent assays (ELISA) were performed to evaluate the binding of Stx2a to C3 and C5. In vitro cytotoxicity assays were performed to evaluate at which Stx2a concentrations and time points the HK-2, HCT-8 and CiGenC cells were still resistant to the cytotoxic effect of Stx2a. Further, cells were stimulated for a suitable time points with varied concentrations of Stx2a, reverse transcription-quantitative PCR (RT-qPCR) was employed to analyse the transcription of these complement components in the cell lines meanwhile ELISAs were performed to analyze their synthesis and release.

Results: Stx2a showed dose dependent binding to C3 and C5 when tested by ELISA. Cytotoxicity assay revealed that HCT-8 cells were significantly more resistant towards Stx2a than CiGenC and HK-2 cells. Any synthesis or release of C3 and C5 has been proved in HK-2 and HCT-8 cell lines after their stimulation with Stx2a, however RT-qPCR analyses revealed an upregulation of their transcription in all three tested cell lines, being most profound in the CiGenC cells.

Conclusion: Stx2a interacts with C3 and C5 and upregulates their gene expression in gut and kidney cell lines providing further evidence to the notion that the complement system is involved in the pathophysiology of eHUS. Whether these findings are advantageous or disadvantageous from a host protection perspective remains to be elucidated.

#### 701-IIP

Intracellular *Staphylococcus aureus* induced cell death and cytokine release in human macrophages

\*J. Bayer<sup>1</sup>, L. Bleul<sup>1</sup>, A. Weber<sup>1,2</sup>, C. Wolz<sup>1</sup>

<sup>1</sup>University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany

<sup>2</sup>University of Tübingen, Interfaculty Institute for Cell Biology, Tübingen, Germany

Professional phagocytes play a key role in the host defense against bacterial pathogens by recognizing, engulfing and eradicating invading bacteria. Additionally they are responsible for the initiation of a proinflammatory immune responses. It was shown that different *S. aureus* factors can lead to apoptosis, pyroptosis and/or necroptosis of macrophages when applied from outside. Little is known about the effects of intracellular *S. aureus* on cell death and immune responses of human macrophages. We followed USA300 JE2 and isogenic mutants after phagocytosis by human macrophages. The fate of bacteria and cells was monitored regarding bacterial survival, escape, membrane damage and cytokine release. We show that two major regulators of *S. aureus* namely AgrABCD and SaePQRS act in concert to facilitate escape of the bacteria after initial uptake by human macrophages. Sae-regulated two-component pore-forming toxins LeukocidinAB (LukAB) and/or Panton-Valentin Leukocidin (PVL) are required for the escape of *S. aureus* from within human macrophages after phagocytosis. Non-toxic strains were able to persist in macrophage without causing cell-death. The intracellular mechanisms of LukAB and PVL induced cell damage and subsequent bacterial escape was independent of canonical apoptosis, inflammasome or lipoproteins. However, toxin dependent IL1 $\beta$  secretion was inflammasome and lipoprotein dependent. Thus, intracellular toxin dependent cell-death and cytokine release are due to different mechanisms.

#### 702-IIP

Identification of specific anti-TPI H8 antibody-epitopes as putative active vaccine against *Staphylococcus aureus*

\*L. Rummler<sup>1,2</sup>, S. Mertins<sup>1,2</sup>, M. Krönke<sup>1,2</sup>, A. Klimka<sup>1,2</sup>

<sup>1</sup>University Hospital of Cologne, Institute for Med. Microbiology, Immunology and Hygiene, Cologne, Germany

<sup>2</sup>German Centre for Infection Research (DZIF), Bonn-Cologne, Germany

Introduction: *Staphylococcus aureus* (*S. aureus*) is a major human pathogen causing clinical infections like bacteremia and endocarditis. The morbidity and socioeconomic burden associated with methicillin-resistant *S. aureus* (MRSA) combined with a lack of new antibiotics defines a clear unmet medical need for novel approaches, including vaccines.

By proteomic approach, we identified vaccine candidates involved in essential metabolic pathways also displayed on the surface of *S. aureus*. Vaccination with triose-phosphate isomerase (TPI), a highly conserved glycolytic enzyme, showed significant protection in our murine sepsis model. After passive vaccination with anti-TPI monoclonal antibodies (moAbs), moAb H8 provided significant higher survival rates compared to other anti-TPI moAbs. Obviously, protection is epitope specific. Using this reverse immunology

approach we identified the H8-TPI-epitope. However, moAb H8 recognizes a discontinuous epitope containing 100 amino acids.

**Objective:** For cost-effective production of an epitope vaccine, the H8-TPI-epitope sequence needs to be shortened without losing its ability to generate anti-TPI H8-like antibodies.

**Materials and Methods:** Peptide-phage display method was applied to identify a mimotope, structurally mimicking the H8-TPI-epitope. After three rounds of biopanning against immobilized anti-TPI H8 antibody, three mimotopes were enriched and analyzed for specific binding to moAb H8. Additionally, based on prior epitope mapping and binding assay results we generated a peptide assumed to contain the discontinuous H8 epitope, called H8-Disco. After immunization of mice with KLH-conjugated mimotopes and H8-Disco, serum was analyzed for the ability to generate TPI-specific antibodies.

**Results:** Three mimotopes specifically bind to moAb H8 in phage ELISA binding assays, and are able to compete binding of moAb H8 to its ligand TPI. However, the mimotopes did not induce TPI-specific antibodies. In contrast, H8-Disco does induce a humoral TPI-specific immune response.

**Conclusion:** H8-Disco could serve as epitope vaccine against *S. aureus*. The protection potential needs to be evaluated in our *S. aureus* mouse infection model.

---

## 703-IIP

### Analysing pathogen-host interaction- Dual RNA sequencing of *Francisella tularensis* infected macrophages

\*M. J. Alber<sup>1</sup>, M. Antwerpen<sup>2</sup>, M. C. Walter<sup>2</sup>, H. von Buttlar<sup>1</sup>

<sup>1</sup>Bundeswehr Institute of Microbiology, Division for Bacteria and Toxins, Munich, Germany

<sup>2</sup>Bundeswehr Institute of Microbiology, Functional Microbiological Genomics and Bioinformatics, Munich, Germany

*Francisella tularensis* (Ft) is a gram-negative, aerobic, non-motile, non-sporulating small coccobacillus that causes the zoonotic disease tularemia. Infection takes place by bites of non-vertebrate vectors like mosquitoes or ticks, by ingestion or via aerosol. *F.t. subspecies tularensis* (Ftt) is highly virulent and highly infective and therefore is in danger to be misused as a bioterroristic or biowarfare agent. In Europe *F.t. subspecies holarctica* (Fth) are mainly less virulent. By classical attenuation live vaccine strains have been generated from F.t.h. During infection, F.t. replicate intracellularly in several cell types i.e. epithelial cells, fibroblasts and hepatocytes but also immune cells like macrophages (MΦs), dendritic cells and neutrophils. Immune mechanisms in infected cells are severely impaired. The interaction of virulence factors from F.t. with cellular components to facilitate this immune evasion is not understood. To investigate this interaction THP-1 derived macrophages were infected with F.t. and a protocol for subsequent staining and sorting of infected cells followed by RNA preparation was optimized. Using dual RNA sequencing the regulation of pathogen and host factors were analysed in macrophages infected with fully virulent or attenuated *Francisella tularensis* strains. Besides others, known virulent factors of F.t. were identified to be upregulated in bacteria replicating in macrophages compared to bacteria growing in axenic medium.

Knowing virulent factors that act in the early phase of infection the development of effective vaccines against F.t. might be possible. Especially factors that enable the pathogen to prevent MHC-II presentation are attractive candidates for such vaccines. Moreover, such virulence factors are candidates for specific therapeutics that can impair *Francisella* growth and help the host immune system to fight the bacterial infection. Enabling MHC-II presentation will lead to IFN-γ production by T-cells to clear *Francisella* from the host organism.

---

## 704-IIP

### Innate Lymphoid Cells in the female genital tract and their role in chlamydial infection

\*S. Barth<sup>1</sup>, S. Kirschnek<sup>1</sup>, G. Häcker<sup>1</sup>

<sup>1</sup>University of Freiburg, Institute for Microbiology and Hygiene, Medical Center, Faculty of Medicine, Freiburg i. Br., Germany

*Chlamydia trachomatis* is the most common bacterial agent of sexually transmitted disease. In women, *C. trachomatis*-infection can cause pelvic inflammatory disease leading to infertility. A mouse model of human *C. trachomatis* infection has been established, where infection with *C. muridarum* in female mice causes comparable symptoms and sequelae.

ILCs (innate lymphoid cells: NK cells, ILC1/2/3) play an important role in tissue defense, repair and homeostasis but little is known about their distribution in the female genital tract and their potential role in genital infections. To get a better insight with respect to immune response, bacterial clearance and tissue damage we infected mice with *C. muridarum*. The results show that in naïve wt mice, NK cells represent the most prominent cell population within the ILCs in the female genital tract. The total NK cell number increases 5.5-fold by 4 days post-infection (dpi), and the cells produce IFNγ. This indicates that they might play a role at early stages of chlamydial infection. At later stages the number of NK cells decreases and reaches baseline levels at 14 dpi. ILC1 and ILC2 cells are also detectable although their total cell numbers in naïve wt mice are much lower than the NK cell number. ILC1 numbers remain stable initially. Intriguingly, until 30 dpi the total cell number of ILC1 cell increases about 10-fold, and there is a correlation of ILC1 number and macroscopically visible tissue damage. This suggests that ILC1s may be involved in tissue damage; whether they play a positive or negative role needs further to be investigated. ILC3s are barely present but appear to convert into ILC1-like cells over the course of infection, as exRORγt-ILC3 cells make up around 17% of total ILC1 numbers 30 dpi. The number of ILC2s does not vary significantly during chlamydial infection at any time. The absolute number of ILCs in neutrophil-deficient-mice is significantly lower 4 dpi. These results suggest that ILCs may partake in anti-bacterial defense and tissue damage, possibly through interaction with myeloid cells.

---

## 705-IIP

*Chlamydia trachomatis* reinfection with different serovars provides serovar-independent protection and faster recovery in a mouse lung infection model.

\*C. Lanfermann<sup>1</sup>, M. Kohn<sup>1</sup>, R. Laudeley<sup>1</sup>, C. Rheinheimer<sup>1</sup>, S. Glage<sup>2</sup>, A. Klos<sup>1</sup>

<sup>1</sup>Hannover Medical School, Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany

<sup>2</sup>Hannover Medical School, Institute for Laboratory Animal Science, Hannover, Germany

**Introduction:** *Chlamydia trachomatis* (Ctr) is an intracellular bacterium. Differences in the major outer membrane proteins



(MOMPs) define serovars, which cause diseases ranging from genital infections with infertility or lung infections in newborns to blinding trachoma. Each year there are 130 Million cases of genital Ctr infections worldwide (WHO). Approx. 4% of female teenagers are infected. Thus, a better understanding of immunological mechanisms of Ctr infection is important.

**Objective:** There are many reports about serovar-specific protection which might hamper the development of a cross-serovar effective vaccine against Ctr. Thus, we wanted to gain precise information about serovar-specific versus a more general protective immunity using Ctr reinfection and combinations of the Ctr serovars D, E, and L2.

**Methods:** In our model, 8 weeks old female C57BL6 mice were infected intranasally with one Ctr serovar. After clinical recovery, 3 and 4 weeks later, potentially remaining chlamydia were eliminated with azithromycin. Reinfection with Ctr was performed 7 weeks after first infection. Body weight and clinical score were determined daily. After 8 days, the anesthetized mice were sacrificed. Subsequently, bacterial load, histology, TNF- $\alpha$ , IFN $\gamma$  and the granulocyte marker Myeloperoxidase were determined in their lungs.

**Results:** After 3-4 days of reinfection mice showed an ameliorated course of disease with faster recovery as compared to controls which had only been mock-infected before. Bacterial load as well as cytokine levels were significantly reduced - independently of the Ctr serovar used.

**Conclusion:** Induced immunity after recovery from Ctr lung infection does not completely prevent reinfection with the identical serovar in mice. Yet, after 3-4 days, the course of infection improves drastically leading to less severe symptoms, a much faster recovery and bacterial clearance. Unexpectedly, a similar degree of cross-protection can be achieved between the three Ctr serovars tested – a promising result in regard of a yet to be developed cross-serovar protecting vaccine.

---

## 706-NRZP

### Susceptibility of invasive *Neisseria meningitidis* strains isolated in Germany towards azithromycin, an alternative agent for post-exposure prophylaxis

\*M. Krone<sup>1</sup>, T. Läm<sup>1</sup>, U. Vogel<sup>1</sup>, H. Claus<sup>1</sup>

<sup>1</sup>Julius-Maximilians-Universität Würzburg, Institut für Hygiene und Mikrobiologie, Würzburg, Germany

## Question

Post-exposure prophylaxis (PEP) to close contacts of invasive meningococcal disease (IMD) cases is recommended in most countries to avoid secondary cases by eradicating supposed meningococcal colonization. Currently, rifampicin, ciprofloxacin, and ceftriaxone are recommended in many countries including Germany. Azithromycin has been shown to eradicate meningococcal colonization. The aim of this study is to assess the azithromycin susceptibility in invasive *Neisseria meningitidis* isolates.

## Methods

A subset of German invasive meningococcal isolates from 2006 to 2018 was selected for this study. Azithromycin minimal inhibitory concentration (MIC) was determined using broth microdilution and agar gradient diffusion.

## Results

Azithromycin MICs as determined by broth microdilution ranged from <0.003 to 2 mg/L (median: 0.50mg/L, Q75: 1mg/L). All isolates were susceptible to azithromycin according to the CLSI breakpoint (95% CI: [0.0%, 1.5%]). There was no significant correlation between MICs determined by broth microdilution and agar gradient diffusion. Nevertheless, both methods were consistent regarding the categorization of all isolates as susceptible.

## Conclusions

Azithromycin is an eligible antibiotic for PEP of IMD close contacts. It is approved for adults as well as children and may even be used in pregnancy. Because of easier application and less toxicity, it might be an alternative to rifampicin and ciprofloxacin, as we found no resistant isolate. Since a gonococcal gene associated with elevated azithromycin MICs has been reported in *N. meningitidis*, careful monitoring of the emergence of resistant strains is nevertheless necessary for meningococci. Lack of concordance of MICs between broth microdilution and agar gradient diffusion needs to be considered.

---

## 707-NRZP

Poliovirus containment in Germany: What labs should know

\*S. Diedrich<sup>1</sup>, K. Keeren<sup>2</sup>, S. Böttcher<sup>1</sup>, F. Feil<sup>3</sup>, K. Beyrer<sup>4</sup>, S. Reiter<sup>5</sup>

<sup>1</sup>Robert Koch-Institut, FG15, NRZ Poliomyelitis und Enteroviren, Berlin, Germany

<sup>2</sup>Robert Koch-Institut, FG15, Office NCC, Berlin, Germany

<sup>3</sup>Ministerium für Soziales, Gesundheit und Gleichstellung, Referat 401, Hannover, Germany

<sup>4</sup>Niedersächsisches Landesgesundheitsamt, Abteilung 2, Infektionsepidemiologie / Meldewesen, Hannover, Germany

<sup>5</sup>Bundesministerium für Gesundheit, Referat 322, Berlin, Germany

To maintain the goal of the Global Polio Eradication Initiative (GPEI) by 2023, containment, which includes biosafety and biosecurity requirements for laboratories, vaccine producers, or other facilities that handle or store eradicated polioviruses (PV) is necessary to minimize facility-associated risk of viruses being released into the community.

Wild poliovirus (WPV) type 2 has been declared eradicated worldwide in 2015. Subsequently the oral vaccine switch from trivalent to bivalent OPV (type 1+3) occurred in 2016. In addition, all PV2 containing materials had to be destroyed / inactivated, unless a lab is certified as poliovirus-essential facility (PEF). To date there is no PEF in Germany.

In late 2019, eradication of WPV type 3 has been confirmed and containment efforts for PV type 3 will focus on WPV / vaccine derived PV (VDPV) and PIM. Containment of OPV3 will follow when type 3 containing vaccines are no longer in use.

In contrast to material known to contain PV, potentially infectious material (PIM), i.e. material at risk for containing PV, is a major challenge. PIM includes stool, respiratory or sewage samples as well as isolates derived from these samples, if the material was collected at a time and place where PV (WPV and vaccine) circulated. Thus, all enteric (rotavirus, bacteria, parasites etc.), respiratory (influenza etc.), nutrition and environmental labs may have a PIM-risk, which needs to be assessed. Appropriate risk mitigation and reporting to national authority has to be done.

To meet future challenges and to form the statutory framework for containment a new paragraph was

implemented into the national "Protection against Infection Act" (IfSG) in Germany in 2017. Conformable to law, all laboratories have to destroy any material containing PV2 and to report to the local public health department any kind of PV material or PIM. By regulation of the Federal Ministry of Health, laboratories will be legally obligated to destroy material containing any of the remaining types of PV according to the progress of the GPEI.

---

## 708-NRZP

### Population Structure of the Genus *Brucella* as Determined by Core-Genome-Based Multi-Locus-Sequence-Typing (cgMLST)

\*H. Scholz<sup>1</sup>

<sup>1</sup>Institut für Mikrobiologie der Bundeswehr, Bakteriologie und Toxinologie, München, Germany

#### Question

The genus *Brucella* comprises various species of both veterinary and human medical importance. Recently, several new species from new hosts, particularly amphibians, had been added to the genus leading to a significant expansion. Previous studies demonstrated that the new species show a much higher genetic diversity than the classical species causing disease in humans, like e.g. *B. melitensis*. In order to get a clear picture of the overall population structure of the expanded genus, we developed a core-genome based MLST (cgMLST) assay which allows accurate positioning of any given strain into a global phylogeographical context.

#### Methods

Creation of a *Brucella* cgMLST typing scheme with SeqSphere+ (Ridom, Germany). Illumina paired-end sequencing of 100 *Brucella* isolates of the in-house strain collection comprising all known species and biovars. New *Brucella* species like *B. microti*, *B. vulpis*, *B. inopinata*, *B. papionis*, and an exclusive set of recently isolated *Brucellae* from amphibians were added to the analysis together with 100 genome sequences from the database (Genbank). A minimum spanning tree (MST) with calculated distances and a neighbor-joining tree were created.

#### Results

The developed assay displayed a robust and accurate population structure of the entire genus. It allows the identification of any given isolate not only at the species but also at the biovar and strain level and therefore can be used for both molecular epidemiological outbreak analysis and source tracking. Atypical *Brucella* species from amphibians showed a higher genetic diversity and were well separated from classical species causing disease in humans. Comparative genome analysis and phylogenetic reconstruction suggest that the highly pathogenic classical species developed from atypical species isolated from amphibians and that the atypical species still have a reservoir in soil.

#### Conclusions

Core-genome-based MLST is a perfect tool to study the overall population structure of *Brucella* but also allows the identification at the strain level during outbreak investigations and source tracking analysis.

## 709-NRZP

### Development of a *Yersinia pestis* cgMLST-Assay for Molecular Outbreak and Forensic Trace-Back Analysis

\*H. Scholz<sup>1</sup>, M. C. Walter<sup>1</sup>, M. Antwerpen<sup>1</sup>

<sup>1</sup>Institut für Mikrobiologie der Bundeswehr, Bakteriologie und Toxinologie, München, Germany

#### Question

Plague, also known as the Black Death, is a life threatening disease caused by the bacterium *Yersinia pestis*. Despite its globally low prevalence (about 1000-3000 human cases each year) and absence in Europe, plague still remains one of the most feared bacterial diseases. Untreated, it might reach a lethality of up to 100%. In an event of an outbreak or intentional release as a biological weapon, source tracking is of utmost importance. We therefore aimed to develop a robust core-genome-based typing assay for the identification of *Y. pestis* at the strain level.

#### Methods

A *Y. pestis* cgMLST typing scheme with SeqSphere+ (Ridom, Germany) was established. Illumina paired-end sequencing of 70 *Y. pestis* isolates of the in-house strain collection including rare strains from Mongolia. Additionally 200 genome sequences from the database (Genbank) comprising all phylogenetic branches of all recognized biovars (*Orientalis*, *Antiqua*, *Medievalis*, *Intermedia*, and *Microtus*) were added to the analysis. Genomes were assembled and a minimum spanning tree (MST) with calculated distances was created with the tools integrated in SeqSphere.

#### Results

The developed assay displayed a robust and accurate population structure of the entire genus based on MLST analysis of 2418 target sequences. *Y. pestis* strains were well separated according to their assigned biovars and phylogenetic branches. The assay allowed the correct assignment of any given isolate to a phylogenetic branch but also enabled the exact identification at the strain level. Because of the highly clonal population structure of *Y. pestis*, strains belonging to the same phylogenetic group were separated by only a few SNPs (alleles)

#### Conclusions

The developed cgMLST assay allows accurate typing at the strain level and therefore represents a perfect tool for molecular epidemiological outbreak- and forensic trace-back analyses. It also can be used to study the overall phylogeographic population structure of the *Y. pestis* including *Y. pseudotuberculosis*.

---

## 710-NRZP

### Report of the National Reference Centre for Multidrug-Resistant Gram-negative Bacteria on Carbapenemases in Germany in 2019

\*N. Pfennigwerth<sup>1</sup>, J. Schauer<sup>2</sup>, L. Marlinghaus<sup>2</sup>, A. Anders<sup>2</sup>, M. Cremanns<sup>2</sup>, S. G. Gatermann<sup>2</sup>

<sup>1</sup>Ruhr-Universität Bochum, Department of Medical Microbiology, Bochum, Germany

<sup>2</sup>Ruhr University Bochum, Department of Medical Microbiology, Bochum, Germany

**Question:** Multidrug-resistance in *Enterobacterales*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of

utmost therapeutic importance since hardly any innovative antimicrobial drug against gram-negative bacteria will be introduced into clinical practice within the next years. Among all resistance mechanisms the worldwide spread of carbapenemases is the most worrisome development. However, the correct identification of carbapenemases is still challenging for the microbiological laboratory.

**Methods:** The National Reference Centre for Multidrug-Resistant Gram-negative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs. All isolates are tested by a wide array of phenotypic and molecular methods. A bioassay based on cell-free extracts and WGS methods allow the detection of still unknown  $\beta$ -lactamases.

**Results:** A total of 5993 isolates were investigated for carbapenemases in the National Reference Centre in 2019 until November 6th. Carbapenemases were found in 1724 *Enterobacterales* strains, 428 of *A. baumannii* and 325 of *P. aeruginosa*. The most frequent carbapenemases in *Enterobacterales* were OXA-48 (n = 468), VIM-1 (n = 292), KPC-2 (n = 184), NDM-1 (n = 163), OXA-244 (n = 135), NDM-5 (n = 134), OXA-181 (n = 67) and KPC-3 (n = 62). OXA-162, OXA-232, VIM-4, GIM-1 and others were found in less than 30 isolates each. In *P. aeruginosa*, VIM-2 was the most frequent carbapenemase (n = 228), followed by GIM-1 (n = 20), GES-5 (n = 12), VIM-1 (n = 11) and VIM-4 (n = 10). IMP-13, IMP-7, NDM-1 and others were found in less than 10 isolates each. OXA-23 was the most frequent carbapenemase in *A. baumannii* (n = 287), followed by OXA-72 (n = 92) and NDM-1 (n = 25). GIM-1, OXA-143, OXA-58 and others were found in less than 10 isolates each.

**Conclusions:** A variety of different carbapenemases is detected in Germany. The molecular epidemiology in Germany differs significantly from observations made in other countries like Greece, Italy or the USA with a predominance of OXA-48. Compared to previous years, variants of OXA-48, especially OXA-244, are again on the rise.

## 711-NRZP

### Microbiological Diagnosis of CF lung infection (the new CF MIQ24 2019)

\*M. Hogardt<sup>1</sup>, B. C. Kahl<sup>2</sup>, S. Besier<sup>1</sup>, J. Steinmann<sup>3</sup>, M. Abele-Horn<sup>4</sup>, E. Richter<sup>5</sup>, G. Jäger<sup>6</sup>, C. Smaczny<sup>7</sup>, L. Sedlacek<sup>8</sup>

<sup>1</sup>University Hospital Frankfurt, Medical Microbiology and Infection Control, Frankfurt Main, Germany

<sup>2</sup>University Hospital Münster, Institute for Medical Microbiology, Münster, Germany

<sup>3</sup>Klinikum Nürnberg, Institut für Klinikhygiene, Medizinische Mikrobiologie und klinische Infektiologie, Nürnberg, Germany

<sup>4</sup>Schrämelstrasse, Munich, Germany

<sup>5</sup>MVZ Labor Dr. Limbach & Kollegen GbR, Institut für Medizinische Diagnostik GmbH Heidelberg, Heidelberg, Germany

<sup>6</sup>Zentrum für Humangenetik und Laboratoriumsdiagnostik, MVZ Martinsried GmbH, Martinsried, Germany

<sup>7</sup>University Hospital Frankfurt, Medizinische Klinik I, Pneumologie und Allergologie, Frankfurt, Germany

<sup>8</sup>University Hanover, Institut für Medical Microbiology and Hospital Epidemiology, Hanover, Germany

Cystic fibrosis (CF) is a multiorgan autosomal recessive genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. A defective CFTR ion channel leads to the formation of viscous secretions and secondary changes in several organs systems. Of note are mainly chronic airway infections with intermittent pulmonary exacerbations and progressive lung function decline. In the nutritious and low-oxygen environments of CF lungs support infections with a

polymicrobial spectrum of pathogens. The highest priority in microbiological routine diagnostics remains the cultural detection of CF-typical pathogens (especially *P. aeruginosa*, *S. aureus*, *B. cepacia*-complex). For a targeted detection of the CF-organisms and adapted pathogen variants, the use of different selective media is required (defined as a basic culture and extended cultural approach). The detection of serum antibodies to *P. aeruginosa* is of diagnostic value for the early detection of *P. aeruginosa* (first lung infection) and of prognostic value for the success of the *P. aeruginosa* eradication therapy. For species identification in CF microbiology MALDI-TOF MS is the method of choice. *B. cepacia* complex species need to be identified by *recA* gene sequencing in case of first isolates. As for chronic airway infection there is little evidence that AST predicts the clinical outcome of CF antimicrobial therapy, indication for AST testing (regarding *P. aeruginosa*) need to be strict (AST is recommended at least four times a year). With the advent of culture-independent approaches (e.g. microbiome analyses) the pathophysiological understanding of the polymicrobial nature of CF lung infection improved. Today molecular methods can complement the routine microbiology e.g. for detection of primary infection with *P. aeruginosa*, and viruses. The poster presentation will focus on the key recommendations of the MIQ24 CF 2019.

## 712-NRZP

### Efficacy of inhalative antibiotic therapy against *P. aeruginosa* in patients with Cystic Fibrosis

T. Burbach<sup>1</sup>, A. Schröder<sup>1</sup>, C. Smaczny<sup>2</sup>, O. Eickmeier<sup>3</sup>, V. Ilievski<sup>1</sup>, S. Besier<sup>1</sup>, \*M. Hogardt<sup>1</sup>

<sup>1</sup>University Hospital Frankfurt, Medical Microbiology and Infection Control, Frankfurt Main, Germany

<sup>2</sup>University Hospital Frankfurt, Medizinische Klinik I, Pneumologie und Allergologie, Frankfurt, Germany

<sup>3</sup>Goethe University Frankfurt, Klinik für Kinder- und Jugendmedizin, Pädiatrische Allergologie, Pneumologie und Mukoviszidose, Frankfurt am Main, Germany

Cystic fibrosis (CF) is characterized by chronic infection and inflammation of the airways. Infection and inflammation lead to progressive lung damage and respiratory failure. Polymicrobial lung infection in CF is dominated by *P. aeruginosa*, which is still the major cause of morbidity and mortality among this population. Repeated antimicrobial treatments of infections and exacerbations are a cornerstone of CF patient management. However, over time multi-resistant variants are increasingly selected. Inhaled antibiotics (intermittent or continuous) are part of the standard care for treating chronic pseudomonas respiratory tract infections. In contrast to systemic therapy (oral or intravenous), inhalation of antibiotics offers the advantage of delivering high drug concentrations to the site of infection with minimal systemic side effects. Aztreonam, colistin, levofloxacin and tobramycin are available for inhalative antibiotic therapy of chronic *P. aeruginosa* CF lung infection. To monitor the microbiological efficacy of inhalative therapy, CF sputum sample pairs before and after an inhalative therapy cycle (typically 28 days) were examined by routine culture (CFU/ml) and quantitative real-time PCR of PMA-treated samples (to detect living cells; CFU equivalent/ml; targeting 16S rDNA and the *P. aeruginosa*-specific genes *regA* and *ecfX*). So far, no significant change (decrease or increase) in the number of *P. aeruginosa* could be shown. In parallel, the activity of colistin (CT), aztreonam (ATM), levofloxacin (LEV) and tobramycin (TOB) against recovered clinical *P. aeruginosa* isolates with variable MIC values (up to  $\geq 1024 \mu\text{g/ml}$  each) was compared in artificial sputum medium (ASM) and by using "in-vivo-like" drug concentrations (500  $\mu\text{g/ml}$ , 1000  $\mu\text{g/ml}$ , 2000  $\mu\text{g/ml}$  for ATM, LEV, TOB and 10  $\mu\text{g/ml}$ , 50  $\mu\text{g/ml}$ , 500  $\mu\text{g/ml}$  for CT). In summary, killing activities are variable but clearly increase

with drug concentration, and show a trend of sufficient killing activity up to MIC values of 128 µg/ml, that may be an first estimate for an inhalative antibiotic breakpoint.

---

### 713-NRZP

Evaluation of *in silico* tools for sequence-based serovar prediction of *Salmonella* spp. at the German National Reference Center

\*S. Banerji<sup>1</sup>, S. Simon<sup>1</sup>, A. Tille<sup>2</sup>, A. Fruth<sup>1</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>Robert Koch-Institute, FG11, Wernigerode, Germany

<sup>2</sup>Robert Koch-Institute, Wernigerode, Germany

**Introduction:** Serotyping is a very important classification method for *Salmonella* species. Several *in silico* serotyping tools for *Salmonella* are available, which are based on whole genome sequence data. The German National Reference Center (NRC) for *Salmonella* and other bacterial enteric pathogens receives around 4,000 *Salmonella* isolates per year.

**Aim:** We assessed the suitability of two selected *in silico* subtyping methods for routine serotype prediction. SeqSero determines the serotype based on the loci for the relevant O- and H-antigens in the bacterial genome by mapping and blast search (Zhang et al. 2015 & 2019). The second method is based on a 7 locus MLST scheme, which looks for allelic differences in 7 conserved housekeeping genes (Achtman et al. 2012) and reveals distinct sequence types (STs).

**Material and methods:** Whole genome sequencing was performed on a MiSeq benchtop sequencer using Illumina's v3 chemistry. Raw reads were submitted to the SeqSero software ([www.denglab.info/SeqSero](http://www.denglab.info/SeqSero)) that was modified for batch analysis. The 7 gene MLST was performed with the Ridom™ SeqSphere+ software where the scheme described above (available from [www.enterobase.warwick.ac.uk](http://www.enterobase.warwick.ac.uk)) was pre-installed.

**Results:** The analysis of 520 *Salmonella* genomes with the two *in silico* typing tools yielded a high correlation with our laboratory serotyping results, i.e. 98% for SeqSero and 95.2% for MLST. SeqSero failed to predict a complete antigenic formula in 5 cases (1%). An additional five cases (1%) of misclassification concerned monophasic strains of *S. Typhimurium* and *S. Choleraesuis*. Serotype prediction based on MLST + Enterobase showed a high error rate for discriminating between monophasic and biphasic variants. The combination of both methods yielded a correlation rate of 99.4% by providing the advantage of both methods.

**Conclusions:** The evaluation of two different *in silico* methods for *Salmonella* serotype prediction showed that both methods highly correlated with laboratory serotyping results. Since both methods complement each other with respect to the provided information, we conclude that a combination of both tools is best suited for routine *in silico* serotyping at the NRC.

---

### 714-NRZP

NGS-based molecular surveillance of infections with *Shigella sonnei*, cgMLST analysis and identified clusters 2018-2019 in Germany

\*A. Fruth<sup>1</sup>, C. Lang<sup>1</sup>, R. Selb<sup>2</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>Robert Koch Institute, Division Enteropathogenic Bacteria and Legionella, Wernigerode, Germany

<sup>2</sup>Robert Koch Institut, Infektionskrankheiten, Wernigerode, Germany

**Question:** Shigellosis is one of the most important diarrheal infections worldwide with estimated 165 million cases per year and is responsible for approximately 60% of death in children less than 5 years of age. There are four known species of *Shigellae*: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. All of these have high transmission rates, while *S. sonnei* is the predominant one. Also 75% of reported cases account for this species in Germany, with an increasing number since 2017. In 2018 surveillance and outbreak detection analysis by pulsed-field gel electrophoresis and phage typing was replaced by NGS-based cgMLST at the NRC. Molecular characterization of *S. sonnei* isolates by cgMLST and performance testing for cluster detection and surveillance implementation was carried out.

**Methods:** In total, 206 isolates of *S. sonnei*, received 2018 and 2019, were characterized at the NRC. Out of these, 120 were under further investigation by Illumina MiSeq whole genome sequencing and Ridom SeqSphere+ v6.0.0 cluster analysis by the utilization of the *Escherichia/Shigella* cgMLST v1 scheme from Enterobase.

**Results:** By molecular characterization, 28 isolates could be connected to the European-wide outbreak strain reported by Public Health England, UK, associated with transmission amongst MSM (1-10 alleles difference). 14 further isolates, originating from a kindergarten outbreak in Berlin in September 2018, were investigated. A cluster of seven isolates was additionally detected, connected with attendance to a music festival in Poland in August 2018, and three additional clusters closely related in cgMLST were investigated in 2018. 9 Clusters could be identified in 2019, including one with a link to an Irish food isolate, one with travel-association to Jordan and one of 9 closely related cases connected with an attendance to a festival in Saxony-Anhalt.

**Conclusion:** cgMLST was successfully implemented at the NRC for surveillance and outbreak detection. Our work confirms that usage of Enterobase *Escherichia/Shigella* cgMLST v1 scheme is qualified for cluster analysis of *Shigella* strains.

---

### 715-NRZP

NGS-based surveillance of *Salmonella enterica* ssp. *enterica* serovar Enteritidis

\*S. Simon<sup>1</sup>, E. Trost<sup>1</sup>, M. Pietsch<sup>1</sup>, A. Flieger<sup>1</sup>

<sup>1</sup>Robert Koch-Institute, Unit for Enteropathogenic Bacteria and Legionella / NRC for *Salmonella* and other bacterial enteric pathogens, Wernigerode, Germany

**Introduction:** Among the more than 2.600 *Salmonella* Serovars, *Salmonella* (S.) Enteritidis is the most prevalent in industrialized countries provoking not only 50% of human *Salmonella* infections in Europe but also causing significant, often multi-national food-borne outbreaks (1). Due to the inherent clonality of this serovar unambiguous case identification and source attribution was often hindered since phenotypic and even molecular subtyping methods had been of insufficient discriminatory power. **Objectives:** *S. Enteritidis* is one of the prioritized organisms for the intensified next generation sequencing (NGS)-based surveillance at the German NRC aiming to enhance the discrimination among certain subtypes defined by conventional subtyping methods. This would result in a more accurate determination of outbreak events and definite identification of the (food) source of infection. **Methods:** All *S. Enteritidis* isolates sent to the NRC underwent phage typing for pre-selection. From strains of the predominant

phage types or from suspected outbreak strains genomic DNA was prepared for Illumina short read sequencing. Raw Reads were analyzed by core genome (cg)MLST (Enterobase scheme in Ridom SeqSphere+ (2,3)) to determine their phylogenetic relationship. **Results:** Since 2016 more than 1.300 *S. Enteritidis* genomes have been analyzed at the NRC. Phenotypically, the strains had been attributed to 26 different phage types. cgMLST revealed 73 clusters (41 of them comprising more than three isolates). Although most clusters consist of only one phage type there is no strict correlation between phage type and cgMLST-based relatedness. Often isolates of the same phage type are more distant from one another than isolates of different phage types indicating that, especially for strains belonging to the predominant phage types, no conclusion can be drawn about their cluster affiliation. **Conclusion:** Applying the high-resolution NGS technique for depicting relationships within the *S. Enteritidis* population gives a comprehensive overview about the circulating lineages and reliably points out developing clusters allowing more focused outbreak investigations and subsequent control measures

## 716-NRZP

### Identification of novel pathogenicity factors in *Bartonella bacilliformis*

\*A. Dichter<sup>1</sup>, S. Garcia Torres<sup>1</sup>, M. Garcia-Quintanilla<sup>1</sup>, S. Becker<sup>1</sup>, J. Berger<sup>2</sup>, W. Ballhorn<sup>1</sup>, V. A. J. Kempf<sup>1</sup>

<sup>1</sup>Universitätsklinikum Frankfurt, Medizinische Mikrobiologie und Krankenhaushygiene, Frankfurt am Main, Germany

<sup>2</sup>Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany

## Introduction:

*Bartonella bacilliformis* is the causative agent of Carrion's disease, a vector-borne biphasic illness restricted to the South American Andes. The pathogen infects human erythrocytes causing severe hemolytic anemia (Oroya fever) with high mortality rates in untreated patients. In a second chronic phase, the infection of endothelial cells results in the formation of blood-filled nodular lesions at skin sites (verruca peruana). Underlying molecular mechanisms of host infection are still unclear. Trimeric autotransporter adhesins (TAAs) play an essential role in bacterial pathogenicity and are encoded in various *Bartonella* spp. We are interested in *Bartonella bacilliformis* adhesins A (BbadA) that has been identified as a TAA.

## Objectives:

The objective of this work is the identification and characterization of immunodominant proteins of *B. bacilliformis* and the genetic and functional characterization of BbadA and Flagellin. Furthermore, potential target proteins will be analyzed for diagnostic and therapeutic use.

## Material & Methods:

Molecular genetic strategies using bacterial mutants (transposon mutagenesis library), recombinant protein expression strategies (heterologous expression library) and a reverse vaccinology approach are used to identify immunodominant proteins and pathogenicity factors of *B. bacilliformis*. Deletion mutants of *bbadA* and *flagellin* were generated using homologous recombination techniques. Furthermore, infection experiments with erythrocytes and endothelial cells will be performed to characterize the role of BbadA in the infection process.

## Results & Conclusion:

A genomic *B. bacilliformis* expression library was established in *E. coli* and screened by using anti *B. bacilliformis* sera. Western blot analysis with reactive mutants revealed immunodominance of recombinant BbadA and Flagellin. The genomic deletion of *bbadA* and *flagellin* was confirmed by PCR and sequencing. Electron microscopy showed the presence of BbadA on the surface of *B. bacilliformis* but not in  $\Delta$ *bbadA*. Furthermore, the deletion of *bbadA* leads to a reduction in cell adhesion to endothelial cells.

## 717-NRZP

### Comparative genomics of *Borrelia* species

\*S. Hepner<sup>1</sup>, J. Hübner<sup>2</sup>, A. Wieser<sup>3</sup>, S. K. Sheppard<sup>4</sup>, E. Mourkas<sup>4</sup>, S. Bayliss<sup>4</sup>, J. Calland<sup>4</sup>, B. Pasoe<sup>4</sup>, E. Rudolph<sup>4</sup>, R. E. Rollins<sup>5</sup>, D. Marosevic<sup>1</sup>, V. Fingerle<sup>1</sup>, G. Margos<sup>1</sup>, A. Sing<sup>1</sup>

<sup>1</sup>Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

<sup>2</sup>Dr. von Hauner Children's Hospital, Munich, Germany

<sup>3</sup>Max von Pettenkofer Institute, Munich, Germany

<sup>4</sup>University of Bath, Bath, United Kingdom

<sup>5</sup>Ludwig-Maximilians Universität, Munich, Germany

The species *Borrelia bavariensis*, *B. garinii* and *B. valaisiana* belong to the *B. burgdorferi* sensu lato complex, but differ in vector/host association and/or human pathogenicity. *Borrelia bavariensis* is adapted to rodents and the species is divided into two populations, one in Eastern Europe and Asia, the other in Western Europe. The population division coincides with the presence of different vectors: *Ixodes persulcatus* in Eastern Europe and Asia, *I. ricinus* in Western Europe. In contrast, *B. garinii* is associated with birds and can use both vectors (*I. persulcatus* and *I. ricinus*). Both *Borrelia* species (*B. bavariensis* and *B. garinii*) are human pathogens, but members of the two *B. bavariensis* populations evoke different manifestations: Patients infected with Asian *B. bavariensis* display mainly skin lesion, patients infected with European *B. bavariensis* often show signs of disseminated manifestation, e.g. neuroborreliosis. In contrast, *B. valaisiana* is not known to be human pathogenic; adapted to birds (like *B. garinii*) and occurs only in Western Europe as it is associated with *I. ricinus*. In this project, we want to compare the genomes of this three *Borrelia* species to identify candidate genes that are involved in adaption and pathogenicity. Cultures of about 30 *Borrelia* strains were initiated and after DNA extraction whole genome sequencing was performed using Pacific Biosciences and Illumina technology. We will use comparative genomics to identify species-specific core and accessory genome and plasmid families. Furthermore, genes present and absent in species with specific adaption (pathogenicity, host/vector adaption) should be identified using the pangenome tool PIRATE. Special attention will be given to the Asian and European subpopulations of *B. bavariensis* to understand the population division and differences in virulence levels. To obtain completed consensus genomes (chromosome and plasmids) we used combined Pacific Biosciences and Illumina data. Initial data indicates that the chromosome, cp26 and lp54 have concordant phylogenies whilst plasmid phylogenies show different clustering of isolates.

## 718-NRZP

### Ten years of laboratory surveillance of the National Reference Laboratory for meningococci and *H. influenzae* (NRZMH): invasive *H. influenzae* disease in Germany 2009-2018

\*T. Lâm<sup>1</sup>, H. Claus<sup>1</sup>, M. Krone<sup>1</sup>, U. Vogel<sup>1</sup>

## Introduction

NRZMHi has been surveying the serotypes and antibiotic resistance of invasive *H. influenzae* isolates since 2008. For this purpose, a representative collection of clinical strains is necessary. The NRL therefore closely collaborates with the public health services and the RKI.

## Objectives

To report on the NRZMHi laboratory surveillance for invasive Hi infections 2009-2018.

## Materials and Methods

Species confirmation and serotyping was done pheno- and genotypically. All isolates were tested by gradient agar diffusion and  $\beta$ -lactamase test. Coverage was assessed by comparing the number of cases processed with the number of notified cases (SurvStat@RKI).

## Results

From 2009 to 2018, about 4200 submissions were processed including invasive specimens from 3919 patients. The NRZMHi identified 3865 viable Hi isolates including 3400 invasive strains. An increase in processed cases was seen in the 10 years observation period with 109 cases in 2009 to 649 in 2018. The estimated coverage rose from 59% (2009) to 75% (2018).

Most invasive isolates were NTHi (2817/3400; 82.9 %), followed by Hif (n=390; 11.5%). Hib (n=95; 2.8%) and Hie (n=86; 2.5%) were less common. The age group most affected was > 40 years old (83.0% of all cases) with 64.8% aged 65+. A high proportion of cases (n=290; 8.5%) was in children aged <5 years, with 4.2% aged < 1 year.

622 isolates (18.2 %) were ampicillin resistant: 67.0 % were  $\beta$ -lactamase+; 33.0% were BLNAR. An increase was observed from 2009 (12%) to 2018 (21%). The cefotaxime resistance was 2% of 1431 tested isolates 2013.

## Conclusions

The NRZMHi is now well established in public health microbiology in Germany, partly due to increased submissions. The dominance of NTHi and its rising trend in the elderly consistent with other countries. NTHi in newborns is rare, but possibly underestimated, which warrants consideration in clinical practice. Hia, which is prevalent in some indigenous populations, was uncommon. The success of the German infant vaccination strategy is confirmed by the reduction of Hib disease. Whilst ampicillin resistance rates in invasive isolates are lower than in respiratory isolates, the rise over years is a matter of concern.

## 719-NRZP

### Discriminative potential of VITEK® MS regarding the genus *Haemophilus*

\*S. Nürnberg<sup>1</sup>, H. Claus<sup>1</sup>, M. Krone<sup>1</sup>, U. Vogel<sup>1</sup>, T. Lâm<sup>1</sup>

<sup>1</sup>Julius-Maximilians-University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany

## Introduction

The distinction between non-typeable *Haemophilus influenzae* (Hi) and *H. haemolyticus* (Hh) is a diagnostic challenge. At the same time, it is clinically relevant, as only Hi is pathogenic. Species identification based on matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) is a well-established diagnostic method. However, validation data on *Haemophilus* spp. are scarce, especially for VITEK MS.

## Objective

Our aim was to investigate the discriminative potential of VITEK MS in the diagnosis of *Haemophilus* spp.

## Materials and Methods

A total of 308 *Haemophilus* spp. isolates including 236 Hi and 51 Hh were analyzed by MALDI-TOF using the VITEK MS (bioMérieux). Strains were derived mostly from the collections of the NRZMHi and the Institute for Hygiene and Microbiology. Species confirmation was done phenotypically and by specific PCRs. In addition, multilocus sequence typing (MLST) was performed on 108 NTHi and 45 Hh isolates to ensure genetic diversity of the strains.

## Results

Phylogenetic analysis based on MLST confirmed the clear separation of Hh and two distinct clusters for Hh and Hi, and represented each species as a clearly separated clade, with profound intraspecies diversity. This finding supported the usefulness of the strain collection and ruled out the possibility that the results might be biased by clonality.

On the genus level, VITEK MS correctly identified all tested isolates. For Hh, 22 of 51 strains were correctly identified, 26 were misdiagnosed as Hi, and 3/51 presented uncertain diagnosis. With regards to the identification of Hi, a sensitivity and a specificity of 100% and 56%, respectively, was calculated.

## Conclusion

VITEK MS currently shows unsatisfactory specificity with regards to the identification of Hi, as many H.h. isolates are misidentified as Hi. An extension of the VITEK® MS reference database is needed to improve its performance.

## 720-NRZP

### Use of whole genome sequencing to analyse of a cluster of invasive meningococcal disease caused by serogroup C in the counties of Ebersberg and Munich (2019)

H. Claus<sup>1</sup>, T. Lâm<sup>1</sup>, M. Krone<sup>1</sup>, \*U. Vogel<sup>1</sup>

<sup>1</sup>Institute for Hygiene and Microbiology, Würzburg, Germany

## Introduction

From March to September 2019 five cases of meningococcal disease occurred in patients aged 13-56 years, which due to the severity of disease and the age group received much media attention and prompted public health officials to initiate a vaccine campaign. We report the genomic characterization of the causative meningococcal isolates.

## Objective

Whole genome sequencing (WGS) of meningococcal serogroup C (MenC) isolates of a spatio-temporal cluster in comparison to other MenC isolates from 2019

## Material and Methods

WGS was done at the Core Unit Systems Medicine of the University of Würzburg on an Illumina NextSeq 500 sequencer using the Nextera XT library preparation. SeqSphere+ software (Ridom) was used for de novo assembly (Velvet) and allele calling for cgMLST assignment. BIGSdb and SeqSphere+ were used for phylogenetic analyses.

## Results

Antigenic finotyping already revealed an identical, but very rare finetype: C: P1.5-1,10-1:F3-6, which otherwise occurred only twice among 6000 isolates collected at the NRZMHI since 2002. The outbreak isolates were assigned to clonal complex (cc) 11. WGS showed that the isolates differed in less than 4 of >1,100 loci from each other. Among all other MenC:cc11 isolates from 2019 (N=4), the next most closely related (N=2) differed in 34 and 163 loci from the Bavarian isolates. They displayed a different antigenic finetype and were isolated in the counties Hamburg and Rhein-Erft. WGS of 119 strains isolated from January through July 2019 demonstrated that the Ebersberg/Munich MenC group of similar strains was the largest of five groups with ten or less differences in core genome loci. The isolates of the other clusters belonged to serogroups B, W and Y, respectively.

## Conclusions

The cluster of IMD in Southern Bavaria was caused by a newly observed clone of cc11, the dominant MenC genotype in Germany. Whilst in this particular case, antigen finotyping already pointed to a unique genotype, cgMLST provided the phylogenetic framework among MenC isolates in Germany.

## 721-NRZP

Results of intensified source finding efforts among community-acquired cases of Legionnaires' disease – first results of the German LeTriWa study; Berlin, 2016–2019

\*C. Lück<sup>1</sup>, U. Buchholz<sup>2</sup>, H. J. Jahn<sup>2</sup>, F. Reber<sup>2</sup>, A. S. Lehfeld<sup>2</sup>, B. Brodhun<sup>2</sup>, W. Haas<sup>2</sup>, C. Förster<sup>3</sup>, B. Schaefer<sup>3</sup>, F. Stemmler<sup>3</sup>, J. Bochmann<sup>3</sup>, Y. Schreiner<sup>3</sup>, C. Gagell<sup>1</sup>, E. Harbich<sup>1</sup>

<sup>1</sup>Med.Fakultät der TU Dresden, Institut Med. Mikrobiologie, Konsiliarlabor Legionella, Dresden, Germany

<sup>2</sup>Robert Koch-Institut, Abteilung Infektionsepidemiologie, Respiratorisch übertragbare Erkrankungen, Berlin, Germany

<sup>3</sup>Umweltbundesamt, Fachgebiet II, 3.5:Mikrobiologie des Trink- und Badewasser, Bad Elster, Germany

**Introduction:** In Germany Legionnaire's disease (LD) is a notifiable form of pneumonia caused by Legionella bacteria. Typically, the bacterium is found in water systems or biofilm, but it must be aerosolized and inhaled, or aspirated to cause disease. The sources of infection of most cases of community-acquired Legionnaires' disease (CALD) are unknown.

**Objective:** Identification of sources of infection of CALD.

Adult cases of CALD reported to district health departments and consenting to the study; age and hospital matched controls.

**Methods:** Questionnaire; standard household water samples analyzed for Legionella concentration followed by subtyping of *Legionella pneumophila* serogroup 1 isolates; among cases further water samples to identify the infectious source as appropriate. Appraisal matrix to identify three source types (external source out of residence, residential non-drinking water source, residential drinking water (DW) as source) using three evidence types (microbiological results, cluster evidence, analytical-comparative evidence (using added information from controls)).

**Results:** Inclusion of 94 study cases and 163 controls. Median age of cases was 68 years (range 25–93 years), 61 were male. We identified an infectious source in 5 cases as confirmed since the same strains (sequence type (ST) 182) were detected in patients and environmental samples. In 12 cases we identified an environmental source as highly probable because strains/clinical samples were positive for the same mabtype. In four cases the suspected environmental source was possibly found, based on mab 3/1 positive strains / samples from patients and water. Cases had a higher chance for the presence of a "Knoxville-like" (mab 3/1 positive strain in their residential DW (OR = 6.02, 95 % confidence interval (CI) 2.0 to 21). However, being a case was not associated with the degree of Legionella concentration in standard household water samples.

**Conclusion:** Using the appraisal matrix we attributed a significant proportion of cases of CALD to an infectious source, predominantly residential DW. Risk for LD seems to be conferred primarily by the type of Legionella rather than the amount.

## 722-NRZP

First case of a non-toxicogenic *tox* gene-bearing *C. ulcerans* blood stream infection in an immunocompromised patient.

\*A. Berger<sup>1</sup>, J. P. Borde<sup>2</sup>, A. Dangel<sup>3</sup>, A. Sing<sup>1</sup>

<sup>1</sup>Bavarian Health and Food Safety Authority, National Consiliary Laboratory for Diphtheria, Oberschleißheim, Germany

<sup>2</sup>Gesundheitszentrum Oberkirch, Oberkirch, Germany

<sup>3</sup>Bavarian Health and Food Safety Authority, Oberschleißheim, Germany

**Introduction** *C. ulcerans* is increasingly recognized as emerging pathogen causing diphtheria-like disease (DLD) and wound infections in many industrialized countries typically related to animal contact. We report the first case of a bloodstream infection caused by a non-toxicogenic *tox* gene-bearing (NTTB) *C. ulcerans* strain in a patient suffering from severe lower back pain. **Objectives** The patient's isolate was characterized and compared with human and veterinary *C. ulcerans* isolates from Germany to find additional virulence factors and to analyze the epidemiological context. **Materials & Methods** Strain identification was performed by biochemical differentiation and MALDI-TOF MS. Susceptibility testing was performed according to CLSI and EUCAST guidelines. Toxicogenicity was verified by real-time PCR and the modified Elek-test. Next generation sequencing analysis was carried out with MiSeq whole genome sequencing and data analysis by cgMLST and virulence factor database searches. **Results** The patient had a medical history of a splenic marginal zone lymphoma. On admission, MR imaging of the total spine showed vertebral osteomyelitis as well as infiltration of the paravertebral tissue with an abscess formation but there was no need for neurosurgical intervention. Three blood culture pairs drawn on admission day grew a NTTB *C. ulcerans* strain. The patient was successfully treated with vancomycin i.v. and afterwards oral doxycycline showing significant decline of sequential CRP values and the resolution of the paravertebral abscesses. The patient completely recovered

after a total duration of therapy of 6 weeks. **Conclusion** A microtrauma was discussed as the most probable source of bacterial colonization and infection in this case. NGS data of the strain revealed a previously described PAI and no prophage was detected. MLST data yielded no close relationship to any other isolate in our database, but sequence type ST326 which we have previously isolated from cats and a fox as well as from a human wound after a cat bite in a woman with DLD. Besides the *tox* gene a variety of virulence genes associated with cell adhesion or iron homeostasis and phospholipase D were detected.

## 723-MIEP

### Detection of CTX-M-15 and AMP-C- beta-lactamases in *Escherichia coli* and *Pseudomonas* species isolates from abattoir and poultry anal and cloacal swab samples in Abakaliki, Ebonyi State Nigeria.

\*I. Iroha<sup>1</sup>, O. Nnaji<sup>1</sup>, B. Oke<sup>1</sup>, P. Ikemsi<sup>1</sup>

<sup>1</sup>Ebonyi State University, Abakaliki, Department of Applied Microbiology, Abakaliki, Nigeria

**Background:** Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam aztreonam. In this study, the presence of ESBL and AMP-C beta lactamase genes were detected in *E. coli* and *Pseudomonas* species isolates of abattoir and poultry origin. **Method:** A total of 300 (poultry n=150 and abattoir n=150) cloacal and anal swabs were collected and analysed using standard microbiology techniques. Isolated bacteria were screened for the presence of ESBL by double disc synergy test while AMP-C detection was by disc diffusion method using cefoxitin and boronic acid. Susceptibility of ESBL producing bacteria to different antibiotics was by disc diffusion method. The presence of antibiotic resistance genes was evaluated using polymerase chain reaction. Clonal relatedness of the isolates was estimated using Random Amplification Polymorphic DNA and further sequenced. **RESULTS:** The results showed that the samples from poultry harbored 42 (28 %) *Escherichia coli* and 5 (3.3 %) *Pseudomonas* species while samples from abattoir harbored 50 (33.3%) *Escherichia coli* only. 12 (28.5 %) and 5 (3.33 %) of *E. coli* from poultry samples and abattoir, respectively were ESBL producers, while 2 (40 %) of *Pseudomonas* species from abattoir were Amp-C producers. ESBL and AMP-C producing isolates were multi-drug resistant. The isolates analyzed by PCR were found to harbor the following antibiotic resistance genes: blaACC-M, blaMOX-M, blaDHA-M, mcr 2, mcr 3, blaCTX-M, blaSHV and blaTEM. Out of 17 isolates screened, 9 co-harbored TEM/SHV genes, 2 CTX-M/SHV genes, 6 CTX-M/TEM genes while 1 co-harbored CTX-M/SHV/TEM; 8 also co-harbored mcr2 /mcr3. RAPD analysis revealed five clusters based on the band patterns observed. DNA sequencing of blaCTX-M-producing isolates revealed the presence of blaCTX-M-15 in most of the ESBL producing *E. coli* from poultry samples. **CONCLUSION:** The presence of these resistance genes in poultry and abattoir is worrisome as it could serve as a potential reservoir and source of spread to humans posing a serious public health threat.

**Keywords:** beta lactamases, ESBL, AMP-C poultry and abattoir.

## 724-MIEP

### Multilocus genotyping of *Giardia duodenalis* in Southwestern Iran. A community survey

\*M. Beirumvand<sup>1</sup>, R. Baghlaninezhad<sup>1</sup>, A. Rafiei<sup>1</sup>, D. Carmena<sup>2</sup>, P. C. Köster<sup>2</sup>, B. Bailo<sup>2</sup>, M. Hernández de Mingo<sup>2</sup>

<sup>1</sup>Ahvaz Jundishapur University of Medical Sciences, Parasitology, Andimeshk, Iran

<sup>2</sup>Parasitology Reference and Research Laboratory, National Centre for Microbiology, Parasitology, Madrid, Spain

**Introduction:** *Giardia duodenalis* is one of the main enteric pathogens associated with diarrheal disease. In developing countries, giardiasis is a major public health concern, particularly in children under five years of age.

**Methods:** Individual faecal specimens were collected from 1,163 individuals (male/female ratio: 0.9; age range 2–75 years) with (n=258) and without (n=905) gastrointestinal symptoms living in rural and urban settings during the period 2017–2018. Conventional methods were used for the initial detection of *G. duodenalis* cysts in faecal specimens. Microscopy-positive samples were confirmed by PCR amplification and sequencing of the small subunit rRNA gene of the parasite. A multilocus genotyping (MLG) scheme targeting the triose phosphate isomerase, the glutamate dehydrogenase, and the beta-giardin genes was used for genotyping purposes.

**Results:** *Giardia duodenalis* cysts were detected in 7.7% (90/1,163) of samples by microscopy, of which 82 were confirmed by ssu-PCR. Successful amplification and sequencing results were obtained for 23.2% (19/82), 9.8% (8/82), and 8.5% (7/82) of the confirmed samples at the *tpi*, *gdh*, and *bg* loci, respectively. MLG data for the three loci were available for two samples only. Out of the 24 samples genotyped at any loci, 50% (12/24) were identified as assemblage A and the remaining half as assemblage B. Overall, AII was the most prevalent sub-assemblage detected (41.7%, 10/24), followed by BIII (25.0%, 6/24), discordant BIII/BIV (5/24) or AII/AIII (2/24) sequences, and BIV (1/24). No significant correlation was demonstrated between a given assemblage/sub-assemblage and the occurrence of clinical symptoms.

**Conclusions:** No genotypes adapted to animal hosts other than humans were found circulating in the investigated human population, suggesting that transmission of human giardiasis in this Iranian region is primarily of anthroponotic nature. Further molecular-based studies are needed to confirm and expand these results, and to ascertain the presence and public health relevance of the parasite in environmental samples.

## 725-MIEP

### Phylogenetically defined isoforms of *Listeria monocytogenes* InlB differently bind receptor gC1qR

\*E. Kalinin<sup>1,2</sup>, Y. Chalenko<sup>2,3</sup>, E. Sysolyatina<sup>2</sup>, K. Sobyatin<sup>2</sup>, S. Ermolaeva<sup>2,3</sup>

<sup>1</sup>Peoples' Friendship University of Russia, Moscow, Russian Federation

<sup>2</sup>Gamaleya Research Centre of Epidemiology and microbiology, Laboratory of Ecology of Infectious Agents, Moscow, Russian Federation

<sup>3</sup>Federal Research Center for Virology and Microbiology, Nizhny Novgorod, Russian Federation

**Introduction** The human and animal pathogen, Gram-positive bacterium *Listeria monocytogenes* has been evolving in at least 4 phylogenetic lineages with predominance of Lineage I and Lineage II strains [1]. Lineage I is predominant among listeriosis in humans while Lineage II is overrepresented among animal and food isolates. The invasion factor InlB presents in all *L. monocytogenes* strains. Phylogenetically defined substitutions in InlB are conservative among strains belonging to a certain phylogenetic lineage. InlB mediates



active invasion into mammalian cells via interactions with tyrosine kinase receptor c-Met, the complement receptor gC1q-R and surface glycosaminoglycans.

**Aim** The aim of study was to establish how different variants of *L. monocytogenes* InlB internalin domain (idInlB) interact with gC1q-R.

**Materials & methods** Size exclusion chromatography were used to characterize idInlBs. Solid-phase microplate binding was used to quantify interactions with gCq1-R. Isogenic recombinant *L. monocytogenes* strains were used to elucidate the input of idInlB isoforms in HEp-2 cell invasion.

**Results** 3 variants of the InlB internalin domain were cloned and purified as His-tag-idInlB. IdInlB9 was typical for Lineage I CC1, idInlB14 is widely spread among Lineage II strains of CC7, CC19, CC20, CC101 etc., idInlB13 is typical for EGDe (Lineage II CC9) strain. In ELISA, idInlB9 bound gC1q-R proteins better than both lineage II idInlBs. Invasion in HEp-2 cells of idInlB9 but not idInlB14-carrying *L. monocytogenes* was gC1q-R dependent.

**Conclusion** Obtained results suggested that interactions of idInlB with gC1q-R are important for virulence Lineage I strains but seem to be of less importance for Lineage II *L. monocytogenes* strains.

**Acknowledgements** This work supported by RSF grant 18-75-00024.

**References** 1. Nightingale, K. K., Windham, K., & Wiedmann, M. (2005). Evolution and molecular phylogeny of *Listeria monocytogenes* isolated from human and animal listeriosis cases and foods. *Journal of Bacteriology*, 187(16), 5537-5551.

## 726-MIEP

Analysis of antimicrobial resistance and virulence in *Staphylococcus aureus* isolates from a community sample

\*I. Kiemann<sup>1</sup>, H. Bednarz<sup>1</sup>, K. Niehaus<sup>1</sup>

<sup>1</sup>University of Bielefeld, Biology - Proteome and Metabolome Research, Bielefeld, Germany

**Introduction:** *S. aureus* is a common colonizer of the human skin and has become a global threat to healthcare. In the last decades, a rising number of *S. aureus* infection cases was observed and isolates with various resistances and enhanced virulence were increasingly found. *S. aureus* is mainly detected in hospitalized patients. As *S. aureus* is a colonizer of the skin, it is assumable that genetic adaption of *S. aureus* happens on healthy carriers.

**Objectives:** Multiresistant *S. aureus* isolates, especially MRSA, are well studied in hospitals. Studies among healthy communities are still rare. In context of the "FlüGe" health study, nasal swabs of refugees were collected. Participants lived in communal accommodations in North Rhine Westphalia, Germany. Nasal swabs were analyzed for presence of *S. aureus* and its provision with antimicrobial resistances and other virulence factors.

**Materials & methods:** *S. aureus* isolates are identified by 16S rDNA sequencing and spa-typing. All isolates are tested for four genes indicating community-acquired (CA) strains. All isolates were tested for antibiotic resistances by microdilution assay. Biofilm formation was investigated by a semi-quantitative biofilm assay.

**Results:** In 161 nasal swabs nine *S. aureus* isolates were identified (5,6%), four were MRSA (2,4%). Isolates belong to common (t084, t304), less common (t021, t991) and rare (t4983) spa-types or have not been described before. In three isolates (33%) *arcA* or *etd* genes indicate CA-isolates. Resistances to penicillin (100%), oxacillin/cefoxitin (44%) erythromycin, clindamycin and tetracycline were found. No resistances to reserve antibiotics were detected. Most strains are able to weakly form biofilms, of which the majority was resolvable by protein kinase, suggesting a protein-based mechanism of biofilm formation.

**Conclusion:** Among refugees, mostly spa-types were identified that are less common in Central Europe, while typical European strains were rarely detected (except for t084). This indicates a co-travelling of the germs with the refugees. Especially rare or newly described strains seem to be less virulent regarding to antimicrobial resistance and biofilm formation.

## 727-MIEP

**Genomic analysis reveals varying degrees of host restriction in *Escherichia coli***

\*S. K. Tiwari<sup>1</sup>, B. Van der Putten<sup>2,3</sup>, T. N. Vinh<sup>4</sup>, M. Bootsma<sup>5</sup>, R. La Ragione<sup>6</sup>, S. Matamoros<sup>2</sup>, N. T. Hoa<sup>4,7</sup>, C. Berens<sup>8</sup>, J. Leng<sup>6</sup>, J. Álvarez<sup>9,10</sup>, D. Jolivet<sup>3</sup>, M. Ferrandis-Vila<sup>3</sup>, J. Ritchie<sup>6</sup>, A. Fruth<sup>11</sup>, S. Schwarz<sup>12</sup>, L. Domínguez<sup>9,10</sup>, M. Ugarte-Ruiz<sup>9</sup>, A. Bethe<sup>12</sup>, C. Huber<sup>13</sup>, V. Johannis<sup>13</sup>, I. Stamm<sup>14</sup>, L. H. Wieler<sup>15</sup>, C. Ewers<sup>16</sup>, A. Fivian-Hughes<sup>6</sup>, C. Menge<sup>8</sup>, C. Schultz<sup>2,3</sup>, T. Semmler<sup>1</sup>

<sup>1</sup>Robert Koch Institute, NG1 Microbial Genomics, Berlin, Germany

<sup>2</sup>Amsterdam UMC, Department of Medical Microbiology, Amsterdam, Netherlands

<sup>3</sup>Amsterdam UMC, Department of Global Health, Amsterdam, Netherlands

<sup>4</sup>Oxford University Clinical Research Unit, Hanoi, Viet Nam

<sup>5</sup>UMC Utrecht, Utrecht, Netherlands

<sup>6</sup>University of Surrey, Department of Pathology and Infectious Diseases, Surrey, United Kingdom

<sup>7</sup>University of Oxford, Nuffield Department of Medicine, Oxford, United Kingdom

<sup>8</sup>Friedrich-Loeffler-Institut, Institute of Molecular Pathogenesis, Jena, Germany

<sup>9</sup>VISA VET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain

<sup>10</sup>Complutense University of Madrid, Department of Animal Health, Faculty of Veterinary Medicine, Madrid, Spain

<sup>11</sup>Robert Koch Institute, Enteropathogenic Bacteria and Legionella, Wernigerode, Germany

<sup>12</sup>Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany

<sup>13</sup>Robert Koch Institute, Advanced light and Electron Microscopy, Berlin, Germany

<sup>14</sup>Vet Med Labor GmbH, Division of IDEXX Laboratories, Ludwigsburg, Germany

<sup>15</sup>Robert Koch Institute, Berlin, Germany

<sup>16</sup>Institute of Hygiene and Infectious Diseases of Animals, Giessen, Germany

**Introduction:** Commensal bacteria, such as *Escherichia (E.) coli*, can serve as reservoir of antimicrobial resistance (AMR) genes in both humans and animals. AMR *E. coli* strains can be transmitted within or between host species and can disseminate resistance genes via horizontal gene transfer (HGT). However, there is a paucity of information regarding which lineages of *E. coli* are adapted/restricted to certain host or if they have lost such specialization, and which genomic determinants contribute to either a narrow or a broad host range.

**Material & Methods:** A panel of 1,198 *E. coli* strains from different hosts (cattle, chicken, pig, human and wild-boar, both healthy and diseased) and geographical locations (Germany, Spain, UK, and Vietnam), isolated between 2003 and 2018 was randomly selected from existing strain

collections and subjected to whole genome-sequencing (Illumina). A maximum-likelihood phylogeny was constructed based on the core genome sequences. The presence of genes enriched in strains isolated from specific host was determined by a bacterial Genome-Wide Association Study (GWAS).

**Results:** The core and accessory genes showed a similar clustering pattern at the population level, indicating a partial host association at the level of sub-populations. We identified adaptation of ST131 and ST117 lineages to human and chicken host respectively. Neither the geographical origin of the strains nor AMR gene profiles were associated with either a specific host or phylogenetic lineage. The GWAS results in *k-mers* associated with chicken, human and cattle hosts, but no *k-mers* association was found for pigs.

**Conclusions:** In general, host association was randomly distributed throughout the entire *E. coli* phylogeny, but various phylogenetic clusters of strains were observed which exhibited a significant enrichment of a specific host. Mapping of *k-mers* significantly associated with a host to reference genomes showed different gene sets enriched in *E. coli* isolates from different hosts. In silico analysis of gene functions revealed a potential role in adaptation to or colonization of different hosts, which may determine broad or narrow host range adaptation.

## 728-MIEP

### Molecular and clinical characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates carrying Panton-Valentine leukocidin in Northern Bavaria, Germany, 2009-2016

\*T. Szumlanski<sup>1</sup>, A. Simbeck<sup>2</sup>, R. Ziegler<sup>1</sup>, T. Holzmann<sup>2</sup>, S. Monecke<sup>3</sup>, R. Ehricht<sup>3</sup>, R. Bertram<sup>4</sup>, W. Schneider-Brachert<sup>5</sup>, J. Steinmann<sup>1</sup>

<sup>1</sup>Klinikum Nürnberg, Institute of Clinical Hygiene, Medical Microbiology and Clinical Infectiology, Nürnberg, Germany

<sup>2</sup>University Hospital Regensburg, Department of Infection Control and Infectious Diseases, Regensburg, Germany

<sup>3</sup>Leibniz Institute of Photonic Technology e.V. Jena (Leibniz-IPHT), Optisch-Molekulare Diagnostik und Systemtechnologien, Jena, Germany

<sup>4</sup>Paracelsus Medical University, Research Department, Nürnberg, Germany

<sup>5</sup>University of Regensburg, Institute of Clinical Microbiology and Hygiene, Regensburg, Germany

**Question:** The spread of community-acquired MRSA (ca-MRSA) carrying Panton-Valentine-leukocidin (PVL) as a distinctive virulence factor is causing recurring and persistent human infections. This study investigated the epidemiology as well as clinical characteristics of PVL-positive MRSA-strains in Northern Bavaria, Germany, over the course of 8 years.

**Methods:** In total, 131 PVL-positive MRSA were collected between 2008-2016 from different hospitals in Northern Bavaria, Germany. Identification was performed by MALDI-TOF, susceptibility testing by VITEK II or agar diffusion. The presence of *mecA* and PVL genes was confirmed by PCR, the isolates were then subjected to genotyping by using a DNA microarray (Alere), and assigned to clonal complexes (CC)/sequence types (ST) accordingly. Furthermore, the molecular characteristics of the isolates were correlated to the clinical presentation of the patients.

**Results:** The predominant strains overall were "ST8-MRSA-IV+ACME" (PVL+), USA300" (27/131; 20,6%), closely followed by "CC30-MRSA-IV (PVL+), Southwest Pacific Clone" (26/131; 19,8%) and "CC80-MRSA-IV (PVL+)"

(25/131; 19,1%). Whereas detection of CC80 showed no clear tendency over the years, the percentage of CC30 per year showed a slight increase. ST8 was declining over the last three years of observation. Other clonal complexes (CC5, CC1, CC772, CC22, CC59, CC88, CC152 and CC93) were detected in smaller numbers. Notably, CC1 (n=7) was not detected before 2015, and clinical data indicate that migration could be a major factor leading to its emergence in this area. The majority (77,9%) of the collected specimens were skin swabs as well as swabs taken from surgical sites. In total, 100 cases (76,3%) were causally linked to an infection (mostly skin and soft tissue) in the respective patients, with the department of dermatology ranking first in terms of samples provided (23/131; 17,6%).

**Conclusion:** This study demonstrated the molecular epidemiology clones of PVL-positive MRSA in Northern Bavaria, Germany. Migration, international travel and evolutionary factors could possibly lead to further diversification and change in the landscape of PVL-positive MRSA in the region.

## 729-MIEP

### Characterisation of a poorly known *S. aureus* /MRSA lineage from the Middle East

\*E. Mueller<sup>1,2</sup>, S. Monecke<sup>1,2</sup>, S. Braun<sup>1,2</sup>, M. Armengol-Porta<sup>3</sup>, M. Bes<sup>4</sup>, S. Boswihi<sup>5</sup>, M. El-Ashker<sup>6</sup>, I. Engelmann<sup>7</sup>, D. Gawlik<sup>8</sup>, H. Hotzel<sup>9</sup>, R. Nassar<sup>10</sup>, A. Reissig<sup>1,2</sup>, A. Ruppelt-Lorz<sup>11</sup>, A. Senok<sup>10</sup>, A. M. Somily<sup>12</sup>, E. Udo<sup>13</sup>, R. Ehricht<sup>1,2</sup>

<sup>1</sup>Leibniz Institute of Photonic Technology (IPHT), Optical Molecular Diagnostics and System Technology, Jena, Germany

<sup>2</sup>InfectoGnostics Research Campus Jena, Jena, Germany

<sup>3</sup>Institute for Clinical Chemistry and Laboratory Medicine, Municipal Hospital Dresden, Dresden, Germany

<sup>4</sup>National Reference Center for Staphylococci, Hospices Civils de Lyon, Lyon, France

<sup>5</sup>Kuwait University, Department of Microbiology, Faculty of Medicine, Jabriya, Kuwait

<sup>6</sup>Faculty of Veterinary Medicine, Mansoura University, Department of Internal Medicine and Infectious Diseases, Mansoura, Egypt

<sup>7</sup>BLINK AG, Jena, Germany

<sup>8</sup>PTC - Phage Technology Center GmbH, Bönen, Germany

<sup>9</sup>Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Institute of Bacterial Infections and Zoonoses, Jena, Germany

<sup>10</sup>Mohammed Bin Rashid University of Medicine and Health Sciences, College of Medicine, Dubai, United Arab Emirates

<sup>11</sup>Medical Faculty "Carl Gustav Carus", Institute for Medical Microbiology and Hygiene, Dresden, Germany

<sup>12</sup>King Khalid University Hospital and King Saud University, Department of Pathology and Laboratory Medicine, College of Medicine, Riyadh, Germany

<sup>13</sup>Kuwait University, Department of Microbiology, Jabriya, Kuwait

While a lot of data on molecular epidemiology of MRSA is available for North America, Western Europe and Australia, much less is known on the distribution and transmission of MRSA clones in the rest of the world. Here, we describe a poorly known lineage from the Middle East, CC1153, to which several distinct strains from humans and from livestock belong.

Isolates were identified during epidemiological studies, during outbreak investigations or when testing for the presence of toxin genes. They were subjected to microarray hybridization and the genome of one isolate from Dubai was sequenced using Nanopore technology.

CC1153 carries *agr* II and capsule type 5 genes. It is positive for *sasG* and most isolates harbor PVL genes. Enterotoxin genes are rarely present (sek, seq). Associated *spa* types include t903 (26-22-19-17-17-20-17-12) and related variants. PVL-positive CC1153-MSSA were found among Egyptian

cattle suffering from subclinical mastitis. It was also identified among humans with skin and soft tissue infections in Saudi Arabia, France and, in one case, Germany. PVL-positive MRSA from that lineage presented with SCCmec V elements that additionally harboured the fusidic acid resistance gene *fusC* or with a previously unknown SCCmec I variant that also included this gene. Differences to the SCCmec I-*fusC* element from the French CC5 Geraldine Clone included an absence of the virulence gene *tirS*. CC1153 MRSA were observed in Arab Gulf countries (Kuwait, United Arab Emirates, Saudi Arabia) and once in a child of Egyptian origin in Germany.

The distribution of CC1153 and the emergence of CC1153 MRSA show the necessity of molecular characterization of *S. aureus* isolates associated with PVL-associated disease or with zoonotic transmission as well as of MRSA that display resistance to fusidic acid. These strains pose a clear and present public health threat as they combine resistance properties to drugs used in hospitals (beta-lactams) as well as to those used topically in the community (fusidic acid). Thus they have selective advantages in either setting and it will be very complicated to contain or to eradicate them once they are established in a population.

---