Springer Spektrum

D13808F · ISSN 0947-0867

www.biospektrum.de

BIOspektrum

Das Magazin für Biowissenschaften

2017

Sonderausgabe

ABSTRACTBOOK

MICROBIOLOGY AND INFECTION 2017

5th Joint Conference of the DGHM & VAAM

VAAM Annual Meeting 2017

69th Annual Meeting of the DGHM

Abstracts

DOI: 10.1007/s12268-017-0772-x

ORAL PRESENTATIONS

Sunday, 05 March 2017

Monday, 06 March 2017

Tuesday, 07 March 2017

Wednesday, 08 March 2017

POSTER PRESENTATIONS

Monday, 06 March 2017 • 16:00-18:00

Postersession 01

Tuesday, 07 March 2017 • 16:00-18:00

Postersession 02

LIST OF ABBREVIATIONS

Special Groups — Oral-/Poster ID

Biotransformation ..... (BTV)
Cyanobacteria ..... (CBV, CPB)
Diagnostics and Quality Assurance ..... (QDV)
Environmental Microbiology ..... (UMV)
Food Microbiology and Food Hygiene ..... (LMV, LMP)
Functional Genomics ..... (FGV)
Fungal Biology and Biotechnology ..... (FBV, FBP)
Identification and Systematics ..... (ISV)
Microbial Cell Biology ..... (MZV)
Regulation and Signal Transduction in Prokaryotes ..... (RSV, RSP)
Symbiotic Interactions ..... (SIV, SIP)

Abstract Topics

Antimicrobial Resistence and Drugs, Infection Prevention ..... (PRV, PRP)
Archaea ..... (ARV, ARP)
Clinical Microbiology and Infectious Diseases ..... (KMV, KMP)
Diagnostic Microbiology ..... (DVV, DVP)
Environmental Microbiology and Ecology ..... (EEV, EEP)
Eukaryotic Pathogens ..... (EKV, EKP)
Free Topics ..... (FTP)
Gastrointestinal Infections ..... (GIV, GIP)
Gene regulation and Non-coding RNA ..... (GRV, GRP)
General and Hospital Hygiene ..... (HYV, HYP)
General Metabolism ..... (GMBV, GMBP)
Genomics and Metagenomics ..... (GMGP)
Infection Epidemiology and Population Genetics ..... (MSV, MSP)
Infection Immunology ..... (IIV, IIP)
Membranes and Transport ..... (MTV, MTP)
Microbial Cell Biology & Cellular Microbiology ..... (MCBV, MCBP)
Microbial Diversity and Evolution ..... (MDEV, MDEP)
Microbial Pathogenesis ..... (MPV, MPP)
Microbiota, Probiota and Host ..... (PWV, PWP)
National Reference Laboratories and Consiliary Laboratories ..... (RKV, RKP)
Phage and CRISPR ..... (PCV, PCP)
Proteomics and Metabolomics ..... (PMP)
Secondary Metabolites ..... (SMV, SMP)
Synthetic Microbiology and Biotechnology ..... (SMBV, SMBP)
Zoonoses ..... (ZOV, ZOP)

DGHM Lecture: The role of the early life microbiome in host metabolic and immunologic development
05 March 2017 • 15:30 – 17:00

001/INV
DGHM Lecture: The role of the early life microbiome in host metabolic and immunologic development
M. J. Blaser*[1]
[1]*New York University Langone Medical Center, New York, United States*

A central feature of mammalian development across an evolutionary timeframe is the high fidelity intergenerational transfer of resident microbes. Yet the developing microbiome, crucial in the early life window of development, has limited resilience, and is vulnerable to perturbation. We have shown that early life antibiotic exposures can alter microbiota development with both metabolic and immunological consequences. We have extended these studies to more fully examine both antibiotic and dietary effects. Network models best explain microbiota dynamics, with linkages to phenotypes. In total, our work highlights the importance of early events in shaping the microbiome of developing animals, with effects that may not be limited to the current generation.

Presentation: Sunday, 5 March 2017 from 15:30 – 17:00 in the Franconia Hall.

Hans-Günter-Schlegel-Lecture (VAAM): Bacterial Small RNAs: What have we learned and what is next?
05 March 2017 • 15:30 – 17:00

002/INV
Hans-Günter-Schlegel Lecture: Bacterial Small RNAs: What have we learned and what is next?
S. Gottesman*[1]
[1]*National Institutes of Health, National Cancer Institute (NCI), Bethesda, Maryland, United States*

Over the last 20 years, it has become clear that many bacteria encode regulatory small RNAs (sRNAs). The major classes of these modulate translation and mRNA stability, both positively and negatively. Methods for identifying these RNAs and their mRNA targets continue to improve, with many of these methods moving from the study of single sRNAs and their targets to global analyses of what sRNAs talk to what mRNAs. Studies on mechanisms of regulation have uncovered unexpected breadth in how such sRNAs and their RNA chaperones can work. Once we have this "parts list", as well as some understanding of the range of outcomes, what is left to learn? This plethora of data leaves us with the challenge of integrating how and when the contribution of these sRNAs is critical for how bacteria respond to hosts, changing conditions, and each other.

Presentation: Sunday, 5 March 2017 from 15:30 – 17:00 in the Franconia Hall.

SHORT LECTURE 01
Physiology of environmental microorganisms (SL EE)
06 March 2017 • 08:30 – 10:00

003/EEV
Outer membrane vesiculation as adaptive stress response in *Pseudomonas putida*
C. Eberlein*[1], F. Klein[1], T. Baumgarten[2], H. J. Heipieper[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Department of Environmental Biotechnology, Leipzig, Germany*
[2]*Center for Biomembrane Research, Department of Biochemistry and Biophysics, Stockholm, Sweden*

Modifications of the cell envelope are one important mechanism of bacteria to deal with environmental stress as well as to cope with toxic hydrocarbons [5,6]. Especially the bacterial cell envelope as complex interface to the environment is very sensitive to stress. Therefore, several mechanisms have been evolved with which bacteria respond to the presence of different environmental stresses. Among these mechanisms, the release of outer membrane vesicles (OMV) in Gram-negative bacteria has gained research interest especially because of its involvement in pathogenic processes such as that of *Pseudomonas aeruginosa* biofilm formation in cystic fibrosis lungs. The role of MV formation as an adaptive response of *Pseudomonas putida* to several stresses and its correlation to biofilm formation was investigated. In the presence of long chain alcohols, high NaCl concentrations, EDTA, and after heat shock cells of this strain release MV very rapidly [4]. The formed MV show similar size and charge properties as well as comparable composition in proteins and fatty acids [3]. Strikingly, the release of MV leads to a dramatic increase in cell surface hydrophobicity as well as to a higher tendency to form biofilms [3]. Thus, cell surface stress in *P. putida* leads to an increased cell surface hydrophobicity by the secretion of MV resulting in elevated biofilm formation [1,3]. This will be discussed as a global mechanism present in all Gram-negative bacteria [1,2].
Acknowledgements: This work was partially supported by the projects BACSIN (Contract No. 211684) and P4SB (Contract No. 633962) of the European Commission.

References
[1] Baumgarten and Heipieper. 2016. Outer membrane vesicle secretion: From envelope stress to biofilm formation. In: de Bruijn F.J. (Ed.) Stress and Environmental Control of Gene Expression in Bacteria. Wiley-Blackwell. Chapter 22.9. in press.
[2] Schwechheimer and Kuehn. 2015. Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. Nat. Rev. Microbiol. 13:605-619.
[3] Baumgarten et al. 2012. Membrane vesicle formation as multiple stress response mechanism enhances cell surface hydrophobicity and biofilm formation of *Pseudomonas putida* DOT-T1E. Appl. Environ. Microbiol. 78:6217-6224.
[4] Baumgarten et al. 2012. Alkanols and chlorophenols cause different physiological adaptive responses on the level of cell surface properties and membrane vesicle formation in *Pseudomonas putida* DOT-T1E . Appl. Microbiol. Biotechnol. 93:837-845.
[5] Heipieper et al. 2007. Solvent-tolerant bacteria for biotransformations in two-phase fermentation systems. Appl. Microbiol. Biotechnol. 74:961-973.
[6] Neumann et al. 2006. Energetics and surface properties of *Pseudomonas putida* DOT-T1E in a two-phase fermentation system with 1-decanol as second phase. Appl. Environ. Microbiol. 72:4232-4238.

Presentation: Monday, 6 March 2017 from 8:30 - 8:45 in the Franconia Hall.

004/EEV

Bacteria on the rocks: Cell architecture and chemotactic behaviour of *Achromatium*

S. Schorn*[1], V. Carvalho[2], H. Cypionka[1]
[1]*Carl von Ossietzky Universität Oldenburg , Institut für Chemie und Biologie des Meeres, Oldenburg, Germany*
[2]*Max Planck Institute for Marine Microbiology, HGF MPG group for Deep Sea Ecology and Technology, Bremen, Germany*

Members of the genus *Achromatium* are the largest freshwater bacteria worldwide. Cells are spherical or cylindrical with average sizes of 15 – 50 µm [1]. *Achromatium* sp. are colorless sulfur-oxidising bacteria and phylogenetically closely affiliate with other large sulfur-oxidisers, such as *Beggiatoa* and *Thiomargarita* [2]. In contrast to the immobile *Thiomargarita*, *Achromatium* exhibits a gliding motility. The most conspicuous feature of *Achromatium* is the presence of intracellular calcium carbonate precipitates ("little rocks"), which fill about 70 % of the cell volume. The function of these calcites is under debate.
In this study we analysed the cell morphology and architecture of *Achromatium* to understand its cellular structures with a main emphasis on the localisation of the large calcite precipitates within the cell. We conducted physiological experiments and fluorescence staining and could reveal that the calcites are not located in the cytoplasm but rather in pockets resulting from invaginations of the cytoplasmic membrane. This conclusion is based on several indications: I. Different treatments of the cells (with dilute hydrochloric acid, ethanol, UV radiation, and bicarbonate wash) rapidly dissolved the calcites without harming the cell shape and motility. II. We visualised intracellular structures using various fluorescent dyes (Nile Red, Cellmask, and Fluorescein) and the cells were analysed via confocal laser scanning microscopy. This confirmed the localisation of the calcites in the periplasm and that the surrounding membrane originated from invaginations of the cytoplasmic membrane. As most of the cell volume was occupied by calcites only little space remained for the cytoplasm and DNA in a separate compartment. This finding sheds new light on the possible role of the internal calcites.
Achromatium thrives in aquatic sediments which are characterised by opposing gradients of oxygen and sulfide. In the second part of our study we analysed how the cells respond to gradients of oxygen and sulfide. Using time lapse microphotography we tracked manually isolated cells upon exposure to oxygen- and sulfide gradients. The cells showed a clear chemotactic response and moved away from both, oxygen and sulfide over several hundred micrometres within an hour. While negative taxis against both oxidising (O_2) and reducing (H_2S) agents might indicate redox taxis, conditions leading to accumulation of the cells at an intermediate redox potential were not yet found.

References

[1] H.-D. Babenzien, F. O. Glöckner, I. M. Head (2005) *Achromatium*. Bergey´s Manual of Systematics of Archaea and Bacteria. 1-8.
[2] V. Salman, T. Yang, T. Berben, F. Klein, E. Angert, A. Teske (2015) Calcite-accumulating large sulfur bacteria of the genus *Achromatium* in Sippewissett Salt Marsh. The ISME journal 9: 2503-2514. Doi:10.1038/ismej.2015.62

Presentation: Monday, 6 March 2017 from 8:45 - 9:00 in the Franconia Hall.

005/EEV

Investigating environmental bacteria by high resolution microscopy and transcriptional profiling: A case study with *Poribacteria*

M. T. Jahn*[1], S. M. Markert[2], T. Ryu[3], T. Ravasi[3], C. Stigloher[2], U. Hentschel[1,4], L. Moitinho-Silva[5]
[1]*GEOMAR Helmholtz-Centre for Ocean Research Kiel, Microbiology, Kiel, Germany*
[2]*University of Würzburg, Division of Electron Microscopy, Würzburg, Germany*
[3]*University of Science and Technology, Thuwal, Saudi Arabia*
[4]*Christian-Albrechts-University of Kiel, Kiel, Germany*
[5]*University of New South Wales, Sydney, Australia*

The majority of environmental microorganisms remains uncultivated and are commonly referred to as microbial dark matter. Here we apply Correlative Light and Electron Microscopy (CLEM) in combination with an RNA-Seq approach to investigate the candidate phylum *Poribacteria*, a prominent and widespread uncultivated bacterial symbiont of marine sponges. We established a protocol for fluorescence *in situ* hybridization-correlative light and electron microscopy (FISH-CLEM) that enabled the identification of poribacterial cells in sponge tissue at electron microscopy resolution. Cellular 3D reconstructions revealed bipolar, spherical structures of low electron density, which are likely carbon reserves. Highly expressed proteins related to cellular compartmentation, were localized in poribacterial cells by combining, to our knowledge, for the first time FISH-CLEM with immunohistochemistry (FISH-IHC-CLEM). Based on our findings, we hypothesize that *Poribacteria* carry out propanediol degradation, atypically localized along the cytoplasmic membrane. The established FISH-IHC-CLEM toolkit effectively combines -omics approaches with functional studies and should thus be applicable in the wider context of microbial ecology.

Presentation: Monday, 6 March 2017 from 9:00 - 9:15 in the Franconia Hall.

006/EEV

Particulate organic matter (POM) degradation in acidobacteria is species-specific

R. L. Hahnke[1], K. J. Huber[2], J. Overmann[2], B. U. Fösel*[2]
[1]*DSMZ, Microorganisms, Braunschweig, Germany*
[2]*DSMZ, Microbial Ecology and Diversity Research, Braunschweig, Germany*

The first two authors contributed equaly to this study.

Acidobacteria are among the most abundant bacterial phyla thriving well especially in terrestrial environments. Based on the first three acidobacterial genomes (Ward *et al*., 2009) it has been postulated that soil acidobacteria function as decomposers participating in the remineralization of plant, fungal, and insect-derived organic matter. Taking into account their high abundance acidobacteria therefore could significantly contribute to the terrestrial carbon cycle. Although the genomic evidence has been corroborated in the recent years, physiological proofs remained fragmentary.
Here we systematically investigated the ability of polymer degradation in *Acidobacteria* subdivision (SD) 4 (class *Blastocatelliia*), 1 (class *Acidobacteriia*) and 6 members. First, exoenzyme production was tested with the commercially available API®ZYM series. Second, substrate degradation was directly assessed on an extensive set of polysaccharides/-peptides (azurin-crosslinked or polymerized with students pigment) in microtiter plate assays (Panschin *et al*., 2016).
The generally high exoenzyme production showed a clear differentiation on subdivision level with the SD4 and 6 members lacking most, if not all, of the sugar cleaving enzymes commonly

expressed by subdivision 1 members. When linking function to phylogeny substrate tests revealed a strain to species specific, exceptionally high degradation potential within the genera *Occallatibacter* (SD1), *Aridibacter* and *Arenimicrobium* (both SD4). From the easier degradable polysaccharides various compounds occasionally were decomposed by SD1, 4 and 6 members. The few "high activity" strains of SD1 and 4 also attacked hemicellulose, barley-β-glucan and α-cellulose. While almost all SD4 and 6 isolates degraded the proteinaceus substrates casein and gelatin, all SD1 members tested lacked this activity. None of the strains degraded chitin, pectin or alginate. In summary, this hints to the potential role of individual acidobacteria as degraders of plant [(hemi)cellulose degradation] rather than fungal and insect (chitin degradation) derived residues.

References

[1] Ward, N. L., Challacombe, J. F., Janssen, P. H. et al. (2009). Three Genomes from the Phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Applied and Environmental Microbiology* **75**, 2046-2056.
[2] Panschin, I., Huang, S., Meier-Kolthoff, J. P.& other authors (2016). Comparing polysaccharide decomposition between the type strains *Gramella echinicola* KMM 6050T (DSM 19838T) and *Gramella portivictoriae* UST040801-001T (DSM 23547T), and emended description of *Gramella echinicola* Nedashkovskaya et al. 2005 emend. Shahina et al. 2014 and *Gramella portivictoriae* Lau et al. 2005. *Standards in Genomic Sciences* 11, 37.

Presentation: Monday, 6 March 2017 from 9:15 - 9:30 in the Franconia Hall.

007/EEV

Volatile-emission of *Serratia plymuthica* 4Rx13 changes due to interaction with *Bacillus subtilis* B2g

M. Kai*[1], L. Thiele[1]
[1]*University of Rostock, Biochemistry, Rostock, Germany*

Bacteria emit enormous amounts of various volatile metabolites. Some of these bacterial volatiles manipulate the growth of other bacteria, but also fungi and plants suggesting that they play important roles in inter-organismic communication. Most of current research was performed on volatiles emitted by isolated and mono-cultivated species neglecting that bacterial phenotype differs when strains are embedded in communities as it is found in natural habitats compared to solitary living or isolated and mono-cultivated strains. The knowledge about volatile emission of bacterial species living in communities, however, is still limited. Here, we show that the structured co-cultivation of *Serratia plymuthica* 4Rx13 and *Bacillus subtilis* B2g in a low-diversity model community grown under nutrient rich conditions led to quantitative changes in volatile emission compared to self-paired mono-cultivations. More precisely, co-culturing revealed a decreased emission of volatiles at the beginning of growth whereas in the later stage the amount of headspace volatiles was increased. Furthermore, one still unidentified compound could solely be detected in co-cultivations. In bioassays *A. thaliana* was stronger inhibited in co-cultivation correlating with the higher amount of volatiles. The result that co-cultivation of bacterial species lead to changes and shifts in the volatile profile and that *A. thaliana* is differentially affected by co-cultivation induced differences of these volatiles demonstrate the importance of considering the effect of microbial communities on volatile emission. Additionally, it provides another approach to discover new volatiles.

Presentation: Monday, 6 March 2017 from 9:30 - 9:45 in the Franconia Hall.

008/EEV

Phenotypic diversity in microbial metabolism

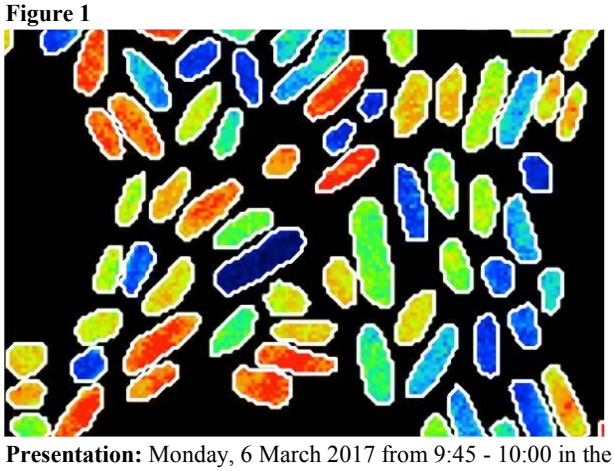
F. Schreiber*[1,2,3], S. Littmann[4], G. Lavik[4], S. Escrig[5], A. Meibom[5,6], M. Kuypers[4], M. Ackermann[2,3]
[1]*Federal Institute for Materials Research and Testing (BAM), Berlin, Germany*
[2]*ETH Zurich, Institute for Biogeochemistry and Pollutant Dynamics, Zurich, Switzerland*
[3]*Eawag, Swiss Federal Institute for Aquatic Science and Technology, Dept. of Environmental Microbiology, Dübendorf, Switzerland*
[4]*Max Planck Institute for Marine Microbiology, Department of Biogeochemistry, Bremen, Germany*
[5]*EPFL Lausanne, Laboratory for Biological Geochemistry, Lausanne, Switzerland*
[6]*University of Lausanne, Center for Advanced Surface Analysis, Lausanne, Switzerland*

Question: Most microorganisms live in environments where nutrients are limited and fluctuate over time. Cells respond to nutrient fluctuations by sensing and adapting their physiological state. Recent studies suggest phenotypic heterogeneity in isogenic populations as an alternative strategy in fluctuating environments, where a subpopulation of cells express a function that allows growth under conditions that might arise in the future. It is unknown how environmental factors such as nutrient limitation shape phenotypic heterogeneity in metabolism and whether this allows cells to respond to nutrient fluctuations.
Methods: We subjected the N2-fixing bacterium *Klebsiella oxytoca* to different levels of substrate limitation and substrate shifts, and obtained time-resolved single-cell measurements of metabolic activities using nanometre-scale secondary ion mass spectrometry (NanoSIMS).
Results: We show that substrate limitation increases phenotypic heterogeneity in metabolism, and this heterogeneity allows cells to cope with substrate fluctuations [1]. We found that the level of NH4+ limitation shapes phenotypic heterogeneity in N2 fixation. In turn, the N2 fixation rate of single cells during NH4+ limitation correlates positively with their growth rate after a shift to NH4+ depletion, experimentally demonstrating the benefit of heterogeneity.
Conclusion: The results indicate that phenotypic heterogeneity is a general solution to two important ecological challenges—nutrient limitation and fluctuations—that many microorganisms face. Currently, we use NanoSIMS to develop a new approach that defines functionally-relevant, phenotypic biodiversity in microbial systems.

References

[1] Schreiber F., Littmann S., Lavik G., Escrig S., Meibom A., Kuypers M.M.M., and Ackermann M. (2016) *Nat. Microbiol.,* 16055.

Figure 1



Presentation: Monday, 6 March 2017 from 9:45 - 10:00 in the Franconia Hall.

SHORT LECTURE 02
Regulatory RNAs in Pathogenic Bacteria (SL GR)
06 March 2017 • 08:30 – 10:00

009/GRV

Identification of regulatory mechanisms important to control *Yersinia pseudotuberculosis* type III secretion and virulence

M. Pimenova*[1], J. Hoßmann[1], I. Vollmer[1], M. Volk[1], R. Steinmann[1], W. Opitz[1], A. K. Heroven[1], P. Dersch[1]
[1]*Helmholtz Centre for Infection Research, Braunschweig, Germany*

The transcriptional regulator LcrF is the major activator of virulence genes required during the ongoing infection stage of *Yersinia*. These virulence factors comprise the *Yersinia* adhesin YadA and the Ysc-Yop type III secretion system (T3SS) with its secreted Yop effector proteins. LcrF expression itself is temperature dependent and occurs only at host temperature (37°C). The 5"-UTR of the *lcrF* mRNA harbours a thermoloop, which prevents ribosome binding to the Shine-Dalgarno (SD) sequence at moderate temperature (25°C). A shift to host temperature results in melting of this structure, enabling *lcrF* translation.
Another environmental signal that further triggers *lcrF* expression is a contact to eukaryotic cells. Moreover, previous data revealed that contact to host cells activates *lcrF* expression even at moderate temperature. This indicates the presence of an unknown regulatory mechanism that enables upregulation of *lcrF* expression upon contact with the host cell in a temperature-independent manner.
Recent data revealed that cell contact-dependent induction of *lcrF* expression requires CsrA. The global post-transcriptional RNA-binding protein CsrA was shown to repress *lcrF* transcription under non-secretion conditions. Sequences in or close to the *yscW-lcrF* promoter region are neccessary for CsrA to repress *lcrF* transcription. Furthermore, CsrA was found to be required for efficient *lcrF* translation under T3SS inducing conditions. Additionally, current experiments highlighted the crucial role of CsrA for the stabilization of the *lcrF* transcript under secretion conditions.
Previous studies demonstrated that expression of T3SS is further controlled by a so-called auto-inhibitory feed forward loop. This mechanism prevents an overproduction of the virulence plasmid encoded genes in the absence of host cell contact. The main regulator of this process is YopD, which together with YopB builds a pore complex in the host cell membrane. In the absence of host cell contact or under non-secretion conditions, YopD accumulates in the bacterial cytoplasm and represses LcrF synthesis. In particular, YopD negatively affects the *lcrF* mRNA stability either directly or via positive regulation of RNA degrading enzymes.

Presentation: Monday, 6 March 2017 from 8:30 - 8:45 in the Barbarossa Hall.

010/GRV

The *Helicobacter pylori* multi-target regulator RepG sRNA activates expression of thioredoxin 2 by direct base-pairing to a short G-rich sequence

S. R. Pernitzsch*[1], T. Bischler[1], K. Förstner[2], B. Aul[1], C. M. Sharma[1]
[1]*Research Center for Infectious Diseases, University of Würzburg, AG Sharma, Würzburg, Germany*
[2]*Research Center for Infectious Diseases & Institute for Molecular Infection Biology, University of Würzburg, Bioinformatics IMIB - ZINF, Würzburg, Germany*

The gram-negative Epsilonproteobacterium *Helicobacter pylori* thrives in the acidic environment of the human stomach, where it can lead to severe inflammation, peptic ulcers, and gastric cancer. In contrast to enterobacteria, little is known about posttranscriptional regulation and small non-coding RNAs (sRNAs) in this widespread human pathogen. Global RNA-sequencing (RNA-seq) identified >60 sRNA candidates in *H. pylori* strain 26695 [1]. Recently, we showed that the highly conserved RepG (Regulator of polymeric G-repeats) sRNA binds with its C/U-rich terminator loop to a variable, homopolymeric G-repeat in the 5" UTR of the mRNA encoding the chemotaxis receptor TlpB [2]. The G-repeat length in the *tlpB* mRNA leader determines the outcome (repression/activation) of RepG-mediated posttranscriptional *tlpB* regulation. This represents an unexpected twist in riboregulation and connects it to phase-variable gene expression control.
Now, we have employed global transcriptome and translatome analyses based on RNA-seq and ribosome profiling, respectively, to identify additional RepG targets. These global approaches revealed that RepG regulates the expression of more than 40 target gene candidates, which are involved in membrane transport, adhesion, LPS modification, and nucleic acid metabolism. Several of the RepG target candidates are important for the colonization of and persistence within the host, implicating a potential role for sRNAs in *H. pylori* virulence. Like the *tlpB* mRNA, most of the additional target mRNAs seem to be targeted at G-rich sequences by RepG. Whereas RepG acts as a repressor on most of the newly identified target genes, expression of the small redox protein thioredoxin 2 (*trx2*) was activated by this sRNA. *H. pylori* uses the thioredoxin system to maintain its thiol/disulfide balance and thus, to survive oxidative stress conditions. Using biochemical and genetic approaches, we demonstrate that RepG affects *trx2* mRNA stability and translation by direct base-pairing interactions to a short G-repeat in the very 5' end of the *trx2* mRNA leader. In line with this, GFP-reporter fusions confirmed that this short G-repeat is sufficient to mediate *trx2* activation through RepG at the transcript and protein level. Overall, our data demonstrate that RepG hijacks a conserved motif of homopolymeric G-repeats and/or G-rich sequences to regulate multiple target genes. Future studies will help to understand how specificity of targeting such G-rich sequences by RepG/sRNAs is achieved.

References

[1] Sharma CM, Hoffmann S, Darfeuille F, Reignier J, Findeiß S, Sittka A, Chabas S, Reiche K, Hackermüller J, Reinhardt R, Stadler PF, Vogel J (2010). *The primary transcriptome of the major human pathogen Helicobacter pylori. Nature*, 464(7286)**:**250-255.
[2] Pernitzsch SR, Tirier SM, Beier D & Sharma CM (2014). *A variable homopolymeric G-repeat defines sRNA-mediated posttranscriptional regulation of a chemotaxis receptor in Helicobacter pylori. Proc Natl Acad Sci USA,* 111(4)**:**E501-10.

Presentation: Monday, 6 March 2017 from 8:45 - 9:00 in the Barbarossa Hall.

011/GRV

sRNAScanner66 influences the expression of pyrimidine metabolism genes in *Streptococcus pyogenes*

R. Pappesch[1], A. Khani[1], S. Mikkat[2], B. Kreikemeyer[1], N. Patenge*[1]
[1]*Universitätsmedizin Rostock, Institut für Medizinische Mikrobiologie, Virologie und Hygiene, Rostock, Germany*
[2]*Universitätsmedizin Rostock, Core Facility Proteome Analysis, Rostock, Germany*

Introduction: *Streptococcus pyogenes* (group A streptococcus, GAS) is a Gram-positive strictly human pathogen. It is responsible for a high number of infections worldwide, ranging from mild superficial infections to invasive life-threatening diseases. The understanding of streptococcal pathogenesis and especially of virulence factor gene regulation is crucial for the development of novel potent therapeutic strategies. Small regulatory RNAs (sRNAs) are known to be important bacterial

modulators of gene regulation. GAS M49 591 (GAS M49) has been screened for sRNA candidate genes with several *in vitro* as well as *in silico* approaches. The sRNAScanner[1] algorithm predicted 137 putative sRNA genes in GAS M49. In this study, we investigated the role of the sRNA candidate sRNAScanner66 in GAS M49.

Materials and Methods: A sRNAScanner66 gene deletion strain (Δ66) was constructed by homologous recombination in GAS M49. For complementation, the deletion strain was transformed with a plasmid carrying the sRNAScanner66 gene (pAT19_66). The ability of the bacteria to adhere to and internalize into human keratinocytes was determined in an antibiotic protection assay. Oxidative stress tolerance was tested by addition of H2O2 to bacterial cultures. *Galleria mellonella* larvae served as invertebrate *in vivo* infection model. Proteome analysis was performed by nanoLC-mass spectrometry. Transcripts were analyzed by RT-qPCR and Northern blotting.

Results: The deletion strain showed reduced adherence to human keratinocytes compared to the wild type strain (WT). Tolerance toward oxidative stress was decreased in Δ66 in comparison to the WT. Furthermore, virulence of Δ66 in a *Galleria mellonella* infection model was attenuated. In the complemented strain, the phenotype was restored in all experiments. Proteome analyses of GAS M49 revealed a differential production of several proteins by Δ66 in comparison to the WT and the complemented strain. Abundances of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase were increased in Δ66. The enzymes catalyze two subsequent steps in UMP *de novo* biosynthesis and play thereby a role in pyrimidine metabolism. Furthermore, the amount of CpsY, a putative transcription factor belonging to the LysR-type transcriptional regulator family, was elevated. In accordance with the protein levels, the transcript levels of the respective genes (*pyrE*, *pyrF,* and *cpsY*) were increased in the deletion strain.

Conclusion: In this study, we observed an influence of the sRNAScanner66 on the regulation of UMP biosynthesis genes. Furthermore, virulence of Δ66 was reduced compared to the WT, even though no dramatic decrease of known virulence factors was observed in the proteome analyses.

[1] Sridhar J, Narmada SR, Sabarinathan R, Ou H-Y, Deng Z, Sekar K, et al. (2010) sRNAscanner: A Computational Tool for Intergenic Small RNA Detection in Bacterial Genomes. PLoS ONE 5(8): e11970. doi:10.1371/journal.pone.0011970

Presentation: Monday, 6 March 2017 from 9:00 - 9:15 in the Barbarossa Hall.

012/GRV

Impact of small regulatory RNA C10 on *Agrobacterium tumefaciens* physiology

J. Borgmann*[1], S. Schäkermann[1], J. Bandow[1], F. Narberhaus[1]
[1]*Ruhr-University Bochum, Microbial Biology, Bochum, Germany*

Small non-coding RNAs are ubiquitous post-transcriptional regulators in all kingdoms of life. In bacteria, the number of identified small regulatory RNAs (sRNAs) has grown rapidly over the past few years. Most of the characterized sRNAs exhibit their regulatory function by base pairing with their target mRNA leading to altered translation or stability of their target [1]. Despite evolving bioinformatic prediction tools the identification of sRNA targets remains a major challenge.

In the model organism *Agrobacterium tumefaciens*, over 650 sRNAs have been discovered indicating a major role of sRNA-mediated regulation in this bacterium [2]. To date, only the two sRNAs RepE, influencing the replication of the Ti plasmid [3], and AbcR1, regulator of various ABC transporters [4], have been functionally characterized.

We report studies of the *trans*-encoded sRNA C10 from the circular chromosome of *A. tumefaciens*. Deletion of C10 causes pleiotropic effects regarding growth, motility, virulence and antibiotic resistance. A combination of proteomics and bioinformatic prediction tools lead to identification of several targets involved in various cellular pathways, confirming the versatile role of C10 in *A. tumefaciens*. Furthermore, we examine the regulation of the sRNA itself by identification of transcriptional regulators.

References

[1] Waters, L.S. & G. Storz (2009) Regulatory RNAs in bacteria. *Cell* 136(4): 615-28.
[2] Wilms, I., Nowrousian, M., Sharma, C.M. & Narberhaus, F. (2012). Deep sequencing uncovers numerous small RNAs on all four replicons of the plant pathogen *Agrobacterium tumefaciens*. *RNA Biol* 9(4): 446-457.
[3] Chai, Y. & Winans, S.C. (2005). A small antisense RNA downregulates expression of an essential replicase protein of an *Agrobacterium tumefaciens*Ti plasmid. *Mol Microbiol* 56(6): 1574–85.
[4] Wilms, I., Voss, B., Hess, W.R., Leichert, L.I. & Narberhaus, F. (2011). Small RNA-mediated control of the *Agrobacterium tumefaciens* GABA binding protein. *Mol Microbiol* 80(2): 492–506.

Presentation: Monday, 6 March 2017 from 9:15 - 9:30 in the Barbarossa Hall.

013/GRV

Two partially redundant sRNA regulators control outer membrane homeostasis in *Vibrio cholerae*

N. Peschek*[1], R. Herzog[1], K. Papenfort[1]
[1]*Ludwig-Maximilians-Universtät München, Faculty of Biology, Biocenter, Munich, Germany*

*Vibrio cholera*e faces drastic environmental changes, when switching from an aquatic lifestyle to host-infection. This biphasic lifestyle requires tight control of membrane integrity and the alternative sigma-factor σ^E, which is strictly required for this process. In gram-negative bacteria, σ^E responds to membrane damage by sensing misfolded outer membrane proteins (OMPs) and off-pathway lipopolysaccharides. In addition to protein synthesis, σ^E induces the transcription of small regulatory RNAs (sRNAs) to repress OMP production and ensure membrane homeostasis.

To identify σ^E-controlled genes, we searched for σ^E-binding sites upstream of transcriptional start sites in the genome of V. cholerae. Using this approach, we discovered a novel σ^E-dependent sRNA, named MicV (mRNA-interfering complementary RNA regulator of OMPs in Vibrio cholerae). We demonstrate that MicV is transcribed as a 68nt sRNA from a σ^E-regulated promoter and responds to various types of envelope stress. MicV targets multiple mRNAs, including ompT to which it binds through a highly conserved, 5'end-located, seed-pairing domain. At the physiological level, MicV functions together with another σ^E-regulated sRNA, VrrA, to collectively control OMP production and membrane integrity.

Presentation: Monday, 6 March 2017 from 9:30 - 9:45 in the Barbarossa Hall.

014/GRV
Dual RNA-seq identifies molecular phenotypes of infection-relevant bacterial non-coding RNAs and RNA-binding proteins
A. Westermann*[1], K. Förstner[1,2], L. Barquist[1], Y. Chao[1], L. Schulte[1], P. Stadler[3], W. D. Hardt[4], J. Vogel[1]
[1]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*
[2]*University of Würzburg, Core Unit Systems Medicine, Würzburg, Germany*
[3]*University of Leipzig, Department of Computer Science & Interdisciplinary Center for Bioinformatics, Leipzig, Germany*

A comprehensive understanding of host-pathogen interactions requires knowledge of gene expression changes in both infection partners. While traditional, probe-dependent approaches like microarrays are insufficient to analyze the full (i.e. coding and non-coding) transcriptome at high resolution, the probe-independent RNA-sequencing (RNA-seq) has begun to revolutionize transcriptomics. Recently, we introduced a new transcriptomics technique referred to as Dual RNA-seq [1, 2] to investigate the interplay of *Salmonella* Typhimurium, an important facultative intracellular pathogen, with human host cells during infection. Here, we harnessed Dual RNA-seq to characterize the *in vivo* significance of *Salmonella* ProQ, a newly discovered bacterial RNA chaperone [3]. Our study revealed the misregulation of motility and virulence genes in the absence of ProQ, but also identified several ProQ-dependent, infection-relevant small regulatory RNAs (sRNAs). We found one of these sRNAs to be processed from the 3UTR of an mRNA in an RNase III-catalyzed cleavage event and to dampen the expression of magnesium importers under conditions mimicking *Salmonella*s intracellular environment. In the infected host cells, MAP kinase signaling and the expression of solute carrier family members was dysregulated upon infection with Δ*proQ* compared to wild-type *Salmonella*. Overall these data indicate how ProQ contributes to *Salmonella* virulence and impacts the mounted host response. Indeed, deletion of *proQ* in *Salmonella* led to their attenuation in various organs of a colitis mouse model. Together, Dual RNA-seq proofs to be a valuable technique for genome-wide host-pathogen interaction studies and to uncover molecular phenotypes [4] for both sRNAs and RNA-binding proteins during infection.

References
[1] Westermann AJ, Gorski SA, Vogel J (2012) Dual RNA-seq of pathogen and host. *Nature Reviews Microbiology*, 10(9), 618-30.
[2] Westermann AJ, Förstner KU, Amman F, Barquist L, Chao Y, Schulte LN, Müller L, Reinhardt R, Stadler PF, Vogel J (2016) Dual RNA-seq unveils noncoding RNA functions in *Salmonella*-host interplay. *Nature,* 529(7587), 496-501.
[3] Smirnov A, Förstner KU, Holmqvist E, Otto A, Günster R, Becher D, Reinhardt R, Vogel J (2016) Grad-seq guides the discovery of ProQ as a major small RNA-binding protein. *PNAS*, 113(41):11591-11596.
[4] Barquist L*, Westermann AJ*, Vogel J (2016) Molecular phenotyping of infection-associated small noncoding RNAs. *Philosophical Transactions of the Royal Society B*, 371(1707).

Presentation: Monday, 6 March 2017 from 9:45 - 10:00 in the Barbarossa Hall.

MINISYMPOSIA 03
Mutualism and manipulation in invertebrate symbioses (FG SI)
06 March 2017 • 08:30 – 10:30

015/SIV
From Omics" to Function: Identification of a Host-Specific Locus in *Aeromonas*
J. Graf*[1]
[1]*University of Connecticut, Molecular and Cell Biology, Storrs, Connecticut, United States*

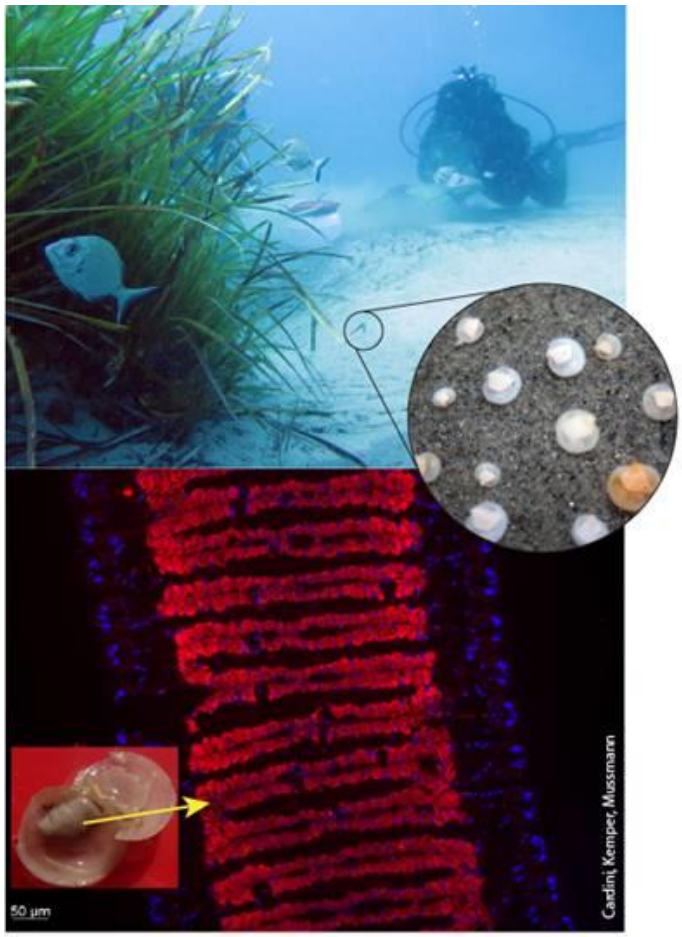
Aeromonas veronii strains can be isolated from a wide range of associations including beneficial ones with leeches and zebra fish and pathogenic ones with human, fish and other animals. Leech isolates appear to better adapted to the leech digestive tract as they outcompete isolates from other sources when competed against each other. A transcriptome from *A. veronii* in the leech gut suggested that early on after the leech consumed a blood meal, *A. veronii* relied on sialic acid as a major nutrient. A genome comparison of over 50 isolates from different sources revealed that all leech isolates contained a sialic acid utilization locus and that this locus was absent in almost all strains obtained from different sources, suggesting that this locus is highly enriched in leech isolates. The distribution of the sialic acid locus among *Aeromonas* strains suggests the horizontal transfer of these genes. Mutants were constructed to assess the role of these genes in colonizing the leech. *nanA,* which is essential for metabolizing sialic acid and *nagAB,* which are essential for metabolizing amino sugars were inactivated. Both mutants had a significant colonization defect and the *nagAB* mutants defect was more dramatic suggesting that additional amino sugars are being utilized. Complementing the *nagAB* mutant with the wild type genes restored its ability to colonize the leech gut. This confirmed the importance of amino sugar metabolism for the growth of leech symbionts in the leech gut as was suggested by transcriptomic and genomic studies.

Presentation: Monday, 6 March 2017 from 8:30 - 9:00 in room 13.

016/SIV
Host-microbe interactions in the wild: Omics-driven insights from marine bivalve symbioses
J. Petersen*[1]
[1]*University of Vienna, Microbiology and Ecosystem Science, Vienna, Austria*
All animals evolved and live today in a countless and immensely diverse "sea" of microbes. Despite this, hundreds of marine animal species have evolved intimate associations with one or a few specific types of chemosynthetic bacteria, which provide them with nutrition. The chemosynthetic symbionts are at least as diverse as their hosts, and have evolved from numerous bacterial lineages multiple times in convergent evolution. "Omics" technologies have helped to usher in a new age of discovery on these so-far uncultivated organisms. Previously, only sulfide and methane were known to power chemosynthetic symbioses, but we recently showed that they can also be fuelled by hydrogen. Carbon fixation by sulfur-oxidizing symbionts is well established, but we have also discovered the genes for nitrogen fixation in the chemosynthetic symbionts of a number of animal hosts. Until now, no nitrogen-fixing chemosynthetic symbiont was known. This discovery raises the intriguing possibility that some chemosynthetic symbionts provide a source of newly fixed nitrogen in the ecosystems their hosts inhabit. "Omics" also promises new insights into the molecular mechanisms of host-symbiont communication. We recently discovered a unique "arsenal" of toxin-like genes, resembling those from pathogens

such as *Yersinia* and *Vibrio*, in the genomes of deep-sea mussel symbionts. We hypothesize that some of these toxin-like genes are involved in molecular communication with their hosts, and others protect their hosts against parasites. Symbioses are usually classified as either "nutritional" or "defensive", therefore, a defensive role for these iconic nutritional symbionts would be surprising. Our ongoing work focuses on chemosynthetic symbioses within the widespread clam family Lucinidae, an ideal system for investigating the mechanisms that underpin host-microbe interactions. We employ a suite of methods including genomics, transcriptomics, proteomics and NanoSIMS to understand the mechanisms of metabolite flux and host-microbe communication in these symbioses.
Figure shows the main focus of our research, the symbiosis between lucinid clams from shallow-water habitats and intracellular sulfur-oxidizing bacteria (red fluorescence in the lower image).

Figure 1



Presentation: Monday, 6 March 2017 from 9:00 - 9:30 in room 13.

017/SIV
Unorthodox transmission modes of endosymbionts in insect hybrids
W. Miller*[1], L. Klasson[2], D. Schneider[1], L. Ehrman[3]
[1]Lab Genome Dynamics, Center of Cell and Developmental Biology, Medical Univ. Vienna, Vienna, Austria
[2]Molecular Evolution, Department of Cell and Molecular Biology, Uppsala University, Uppsala, Sweden
[3]Natural Sciences, State University of New York, Purchase College, Purchase, New York, USA

Although not a new idea, recent studies suggest that differences in the composition of symbiotic microbes between hosts can lead to reproductive isolation, and as a consequence also to speciation. Despite the fact that microbes are universally present in eukaryotes, they are rarely considered as a driving force of speciation, and chances are their contribution to speciation is overlooked. Symbiotic bacteria of the genus Wolbachia are known to affect their hosts'reproduction in adaptive manners to improve the propagation of the maternally transmitted endosymbiont throughout populations. These reproductive alteration that can result in postmating isolation via cytoplasmic incompatibilities, have recently been shown to foster also premating isolation in some host---symbiont associations such as the Drosophila paulistorum species complex, giving even more reason to assume that Wolbachia can play a significant role in host speciation.

Here we will present most recent data on the involvement of Wolbachia in host speciation in Neotropical Drosophila species groups that are under incipient speciation in nature, carrying closely related but incompatible Wolbachia strains. We will also show that naturally incompatible and sterile interspecies hybrids can be rescued by means of mild paternal Wolbachia-knockdown before forced mating, giving rise to fertile progeny and thereby stable hybrid linesvia sib mating. Even more surprisingly, such rescued hybrid lines show complete sexual isolation to their parental lines plus unambiguous signatures ofpaternal inheritance of both their cytoplasmic endosymbionts, i.e., of mitochondria and Wolbachia.

Presentation: Monday, 6 March 2017 from 9:30 - 10:00 in room 13.

018/SIV
The symbiosis of Blochmannia with carpenter ants: Immune tolerance and vertical transmission
M. Kupper*[1]
[1]Uni Würzburg , Mikrobiologie, Würzburg, Germany

Symbioses with g-Proteobacteria of the genus *Blochmannia* may have at least partially facilitated the evolutionary success of ants of the genus *Camponotus*, members of which are found worldwide in virtually all terrestrial habitats. *Blochmannia floridanus,* the endosymbionts of the Florida carpenter ant *Camponotus floridanus*, resides free in the cytoplasm of so-called bacteriocytes, which are intercalated in between midgut tissue cells. The symbionts genome sequence and feeding experiments with *C. floridanus* provided strong evidence that the endosymbionts enrich their host`s diet with essential amino acids. During metamorphosis, the number of bacterial endosymbionts and bacteriocytes is strongly increasing suggesting a contribution of *B. floridanus* to its host`s biology via nutritional upgrading especially during metamorphosis. The endosymbionts are vertically transmitted to the progeny and an early infestation of determined oocytes within the germarium of the polytrophic-meroistic ovarioles of the ant hosts could be observed. In contrast to other ant species and Hymenoptera, the bacteria are exclusively transmitted from follicle cells into the growing oocytes probably involving endocytotic processes, while nurse cells are never infected.

Despite its essential role and its long-lasting integration into the host`s biology for Millions of years, *B. floridanus* is still recognised by the hosts immune system and induces a significant immune response. The full transcriptome analysis of immune-challenged and control animals revealed the presence of a broad immune gene repertoire although relatively few peptidoglycan recognition proteins (PGRPs) and known antimicrobial peptides could be detected. Interestingly, the expression levels of genes involved in bacterial recognition and antimicrobial activity are significantly lower in the midgut tissue than in the residual body especially during pupation when the bacteria massively replicate. In contrast, two negative immune regulators, the amidase PGRPs PGRP-LB and PGRP-SC2, are highly expressed during pupation. These amidase PGRPs are known to down-modulate the immune response by cleaving bacterial peptidoglycan. High expression levels of these PGRPs as well as of tollip, a negative regulator of the Toll signalling pathway, were also observed in active ovaries of *C. floridanus* workers. Consequently, it can be assumed that a down-modulation of immune response in midguts and ovaries of the ants supports tolerance and transmission of the bacterial endosymbionts and therefore the maintenance of the symbiosis.

Presentation: Monday, 6 March 2017 from 10:00 - 10:30 in room 13.

SHORT LECTURE 04
Membranes and Transport (SL MT)
06 March 2017 • 08:30 – 10:00

019/MTV

Pyruvate Sensing and Transport in *Escherichia coli*

I. Kristoficova*[1], S. Behr[1], M. Wittig[2], C. Vilhena[1], P. Schmitt-Kopplin[2], M. Hadjifrangiskou[3], K. Jung[1]
[1]*LMU, Microbiology, Munich, Germany*
[2]*Helmholtz Zentrum München, Neuherberg, Germany*
[3]*Vanderbilt University Medical Center, Departments of Pharmacology,, Nashville, United States*

Escherichia coli is able to adapt and to cope with nutrient perturbations by regulating genes involved in metabolism. Pyruvate plays an important role in the carbon metabolism of *E. coli* and other enteric bacteria. It forms the central node of carbon catabolism, energy metabolism and anabolism.
The BtsS/BtsR two component system of *E. coli*, formerly known as YehU/YehT-system, contributes to carbon scavenging. The system belongs to the LytS/LytTR family and regulates the expression of *yjiY* encoding a transporter belonging to the CstA superfamily and APC superfamily (1). This system is found in all commensal and pathogenic representatives within the *Enterobacteriaceae.*
We found that activation of BtsS/BtsR is dependent on nutrient starvation and the extracellular availability of pyruvate. Various in vitro assays confirmed that BtsS is a high affinity sensor of extracellular pyruvate (2).
Then we focused on the identification of the function of the transport protein YjiY. YjiY contains 18 transmembrane helices, and no representative of the CstA family has been characterized thus far. Transport studies with intact cells, right-side-out membrane vesicles provided first evidence that YjiY is a transporter for pyruvate. Finally, reconstitution of the purified YjiY into proteoliposomes revealed that YjiY functions as specific transporter for pyruvate which is driven by the proton motive force.
Taken together, we present evidence that the histidine kinase BtsS responds to extracellular pyruvate under nutrient limitation and leads to expression of *yjiY*, encoding a pyruvate transporter. YjiY is the first pyruvate transporter described for *E. coli*.

References

[1] Kraxenberger, T., Fried, L., Behr, S., Jung, K. (2012) First Insights into the Unexplored Two-Component System YehU/YehT in *Escherichia coli*. *Bacteriol*. 2012;194:4272-4284.
[2] Behr, S., Kristoficova, I., Witting, M., Breland, E., Eberly, A., Sachs, C., Schmitt-Kopplin, P., Hadjifrangiskou, M., Jung, K., Identification of a High-Affinity Pyruvate Receptor in *Escherichia coli*. Sci. *Rep.,* submitted

Presentation: Monday, 6 March 2017 from 8:30 - 8:45 in room 10-11.

020/MTV

Lantibiotics as an antibiotic alternative: Can we bypass the natural resistance systems in human pathogens?

S. Smits*[1], J. Zaschke[1], R. Clemens[1], S. Khosa[1]
[1]*Heinrich Heine University, Institute of Biochemistry, Duesseldorf, Germany*

Antibiotic resistance is a major focus of medical research throughout the world. Lantibiotics are small antimicrobial peptides possessing high potency and specificity, and thus, are considered to be an excellent and novel candidate as an alternative to antibiotics. Their high activity is reflected by the observation that already 2-4 nM of the lantibiotic nisin is enough to kill a *L. lactis* culture. However, some human pathogenic strains are inherently resistant against these lantibiotics due to the up-regulation of a gene cluster (Khosa 2013). We focused on this gene cluster from the human pathogenic bacteria S. *agalactiae* that confers resistance against the model lantibiotic nisin. This gene cluster encodes for 4 functional proteins: the membrane-anchored nisin resistance protein *Sa*NSR, the ABC transporter *Sa*NsrFP and the two-component system comprising of *Sa*NsrR and *Sa*NsrK (Khosa 2016a).
*Sa*NSR represents a belongs to the C-terminal specific protease superfamily, which cleaves the substrate nisin, thereby, lowering its antimicrobial activity. We solved the high-resolution structure of *Sa*NSR (Khosa 2016b). Using mutagenesis *in vivo* assays and molecular modeling, we were able to identify that *Sa*NSR recognizes exclusively the C-terminus of nisin. Thereby highlighting that *Sa*NSR is a lantibiotic specific protease.
the ABC transporter *Sa*NsrFP belongs to the CprP superfamily identified in *C. difficile* shown to be involved in lantiobitic resistance. *Sa*NsrFP has broad substrate specificity and recognizes the N-terminal part of nisin. Since this part is structurally conserved in other lantibiotics such as gallidermin and subtilin we tested these lantibiotics as well and could show that NsrFP also confers resistance against these lantibiotics. *Sa*NsrFP is able to confer a 6-8 times resistance against nisin as determined by IC50 growth assays. Here the cells still grow after the addition of 60 nM nisin where as cells lacking the transporter only can deal with 4-8 nM. Interestingly, mutants of nisin are better recognized by *Sa*NsrFP reflected in up to 130 fold resistance observed.
*Sa*NSR and *Sa*NsrFP together contribute towards the high resistance of *S. agalactiae* against nisin and other lantibiotics. Here, we will highlight our latest efforts towards (I) understanding the exact molecular mechanism of lantibiotic resistance as well (II) our recent advances in identifying inhibitors for these proteins using a a large pharmaceutical compound databank.

References

[1] Khosa, S., et al (2016a) Plos One Mar 1;11(3):e0149903
[2] Khosa S. et al (2016b) Scientific Reports 6, 18679
[3] Khosa, S., et al (2013). *Biological chemistry* 394, 1543-1549

Presentation: Monday, 6 March 2017 from 8:45 - 9:00 in room 10-11.

021/MTV

Pharmacology of Binary Toxins: Blockage of Channel Formation *in vitro* and Intoxication in Cell Based Assays by Heterocyclic Compounds

C. Beitzinger[1], A. Kronhardt[1], H. Barth[2], R. Benz*[3]
[1]*University of Würzburg, Rudolf-Virchow-Center, Würzburg, Germany*
[2]*University of Ulm, Institute of Pharmacology and Toxicology, Ulm, Germany*
[3]*Jacobs University, Life Sciences and Chemistry, Bremen, Germany*

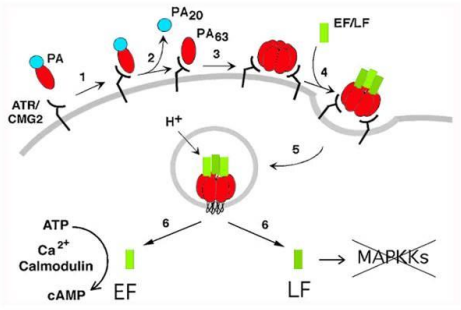
Background: Several potent intracellular acting bacterial protein toxins of the AB-type, which are known to enter cells by endocytosis, are shown to produce channels. This holds true for protective antigen (PA), the binding component of the tripartite anthrax-toxin of *Bacillus anthracis,* and the binding component C2IIa of C2-toxin of *Clostridium botulinum*. Evidence has been presented that translocation of the enzymatic components of the toxins across the endosomal membrane of target cells and channel formation by the heptameric PA_{63} and C2IIa binding/translocation components are related phenomena (see Fig. 1). Chloroquine and some 4-aminoquinolones, known as potent drugs against *Plasmodium falciparium* infection of humans, block efficiently the PA_{63}- and the C2IIa-channels in a dose dependent way. Similarly, they inhibit intoxication of living cells, such as Vero cells.
Methodology/Principal Findings: Here we demonstrate that related positively charged heterocyclic aminoquinolinium salts block the C2IIa- and the PA_{63}-channel in the µM range, when both, inhibitor and the binding components PA_{63} and C2IIa are added to the same side of the membrane, the cis-side, which corresponds to the lumen of acidified endosomal vesicles of target cells. Noise-analysis allowed the study of the kinetics of the plug formation by the heterocycles. Similarly, we investigated the effect of the chloroquine analogues for binding to PA_{63}- and C2IIa-channels on intoxication of Vero cells using Anthrax and C2-toxin, which suggested indeed that these compounds blocked intoxication with much higher efficiency than chloroquine.
Conclusions/Significance: These results strongly argue in favor of a transport of the enzymatic components of the binary anthrax- and C2-toxins through the channels formed by the binding components and suggest that the heterocycles used in this study could represent attractive candidates for development of novel therapeutic strategies against intoxication with anthrax- and C2-toxin.

References

[1] Barth H, Aktories K, Popoff MR, Stiles BG, Binary bacterial toxins: Biochemistry, biology and application of common *Clostridium* and *Bacillus* proteins, Micro. Mol. Biol. Rev. (2004) 68, 373-402.
[2] Kreidler AM, Benz R, Barth H. Chloroquine derivatives block the translocation pores and inhibit cellular entry of *Clostridium botulinum* C2 toxin and *Bacillus anthracis* lethal toxin. Arch Toxicol. (2016) Apr 22. [Epub ahead of print]
[3] Kronhardt A, Beitzinger C, Barth H, Benz R. Chloroquine Analog Interaction with C2- and Iota-Toxin in Vitro and in Living Cells. Toxins (Basel). (2016) Aug 10;8(8).

Fig. 1. Translocation of the anthrax enzymatic components edema factor (EF) and lethal factor (LF) through the protective antigen (PA_{63}) channel into the cell.
1. Receptor binding of PA. 2. Activation of PA by proteases to yield PA_{63}. 3. Formation of the PA_{63} prepore. 4. Binding of the enzymatic components EF and LF to the prepore. 5. Endocytosis of the prepore-enzymatic components complex. 6. Release of EF and LF into the cytosol following acidification of the endosome.

Figure 1



Presentation: Monday, 6 March 2017 from 9:00 - 9:15 in room 10-11.

022/MTV

Carbohydrate uptake in *Advenella mimigardefordensis* strain DPN7^T is mediated by periplasmic sugar oxidation and a TRAP-transport system

C. Meinert*[1], A. Steinbüchel[1]
[1]*Westfälische Wilhelms-Universität, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*

Substrate binding proteins (SBP) of primary and secondary transporters enable high-affinity uptake of solutes in bacteria. In this study, we investigated an SBP (DctP$_{Am}$) of a tripartite ATP-independent periplasmic transport system (TRAP) in *Advenella mimigardefordensis* strain DPN7^T. Deletion of *dctP*$_{Am}$ as well as of the two transmembrane compounds of the tripartite transporter, *dctQ* and *dctM*, impaired growth of *A. mimigardefordensis* strain DPN7^T, if cultivated on mineral salt medium supplemented with D-glucose, D-galactose, L-arabinose, D-fucose, D-xylose or D-gluconic acid, respectively, as sole carbon sources. The wild type phenotype was restored during complementation studies of *A. mimigardefordensis* Δ*dctP*$_{Am}$ when the broad host range vector pBBR1MCS-5::*dctP*$_{Am}$ was used. Furthermore, an uptake assay with radiolabelled [$_{14}$C(U)]-D-glucose clearly showed that the deletion of *dctP*$_{Am}$, *dctQ* and *dctM*, respectively, disabled the uptake of this aldose in cells of either mutant strain. Determination of the dissociation constant K_D performing thermal shift assays showed, that DctP$_{Am}$ did not interact with the the above-mentioned sugar molecules. However, a shift in the melting temperature of the protein in the presence of D-gluconic acid was observed. Therefore, the interactions of DctP$_{Am}$ with D-glactonate, D-fuconic acid and D-xylonic acid were examined and the shift in melting temperature could also be determined. These results led to the assumption that the sugar molecules are oxidized prior to transport and that no other carbohydrate transport system for D-glucose, D-xylose, D-galactose, D-fucose and most probably L-arabinose exists in *A. mimigardefordensis* strain DPN7^T. The strain possesses three membrane-bound quinoprotein glucose dehydrogenases, which most likely oxidize the sugar molecules in the periplasm prior to transport.

Presentation: Monday, 6 March 2017 from 9:15 - 9:30 in room 10-11.

023/MTV

Helical jackknives control the gates of the double-pore K$^+$ transporter KtrAB

I. Hänelt*[1], M. Diskowski[1], A. R. Mehdipour[2], D. Wunnicke[1], D. J. Mills[2], V. Mikusevic[1], G. Hummer[2], J. Vonck[2]
[1]*Goethe University Frankfurt, Frankfurt a.M., Germany*
[2]*Max Planck Institute of Biophysics, Frankfurt a.M., Germany*

Question: KtrAB and TrkAH are nucleotide-regulated K^+-translocating systems crucial for bacterial survival. As osmoregulators, these transmembrane protein complexes are

responsible for volume and pH homeostasis, and have been implicated as pathogenicity factors. We aimed at answering how the systems sense hyperosmotic stress conditions and by which mechanisms potassium ion gating is achieved.
Methods: We have used cryo-electron microscopy for structural determination. Mechanistic and dynamic insights were achieved by combining the structural data with pulsed EPR spectroscopy and molecular dynamics simulations. The gained molecular data were complemented with functional approaches like growth and transport assays.
Results: We report the ADP-bound structure of KtrAB from *Vibrio alginolyticus*, determined by cryo-electron microscopy at 6.6 Å resolution, and propose a novel regulatory mechanism. Exchange of activating ATP to inactivating ADP triggers a completely unexpected conformational change: Short helical segments in the K^+-translocating KtrB dimer organize into two long helices that penetrate deeply into the regulatory KtrA ring to connect nucleotide binding sites and ion gates.
Conclusions: Our study ends a long-standing controversy about the conformation of the inactive KtrAB system and explains how ligand-induced action is achieved at a distance. The helical jackknives operating the gates of the double-pore KtrAB transporter describe a mechanism very different from single-pore RCK channels, which sheds new light on potassium channel gating in general. We hypothesize that with the knowledge of the inactive complex structure-guided drug development can be advanced which could result in an entirely new line of antibiotics.

Presentation: Monday, 6 March 2017 from 9:30 - 9:45 in room 10-11.

024/MTV
Biochemical characterization of the MOB_H relaxase TraI from *Neisseria gonorrhoeae*
J. Heilers*[1], E. M. Heller[2], J. Koch[2], S. Smits[3], C. van der Does[1]
[1]*Albert-Ludwigs-University Freiburg, Freiburg im Breisgau, Germany*
[2]*Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany*
[3]*Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany*

Introduction: Type-IV-Secretion Systems (T4SS) are multiprotein membrane spanning complexes that transport DNA or protein substrates to bacterial or eukaryotic target cells. T4SS are subdivided into effector-translocator systems, conjugation systems and the small group of DNA-release and uptake systems. The latter group includes the T4SS of *Neisseria gonorrhoeae*, the only known DNA release system. An essential protein for conjugative DNA transfer and for the DNA release by *N. gonorrhoeae* is the relaxase, a multi-domain protein with the name-giving relaxase domain at its N-terminus. Relaxases are HUH-endonucleases that cleave DNA strands within the origin of transfer (*oriT*) region. Thereby a ssDNA transfer intermediate is produced, which is then targeted to the secretion apparatus. In the cleavage reaction relaxases form a covalent phosphotyrosyl linkage with the DNA in a metal-dependent manner.
The relaxase of *N. gonorrhoeae* belongs to the large but mainly uncharacterized MOB_H-family of relaxases. A unique feature of MOB_H relaxases is the presence of two putative metal-binding motifs. The first motif is an alternative HHH-domain, which differs from the common HUH-motif present in many other relaxases. The second motif is a HD-domain, which usually is associated with proteins that exhibit a phosphohydrolase activity. Furthermore, most MOB_H-relaxases have a C-terminal DUF1528 which is suggested to be involved in protein-protein interactions.
Methods and Results: We purified TraI as full-length protein (FL-TraI) and the relaxase domain seperately. FL-TraI elutes as a dimer from size exclusion chromatography whereas the relaxase domain elutes as a monomer. With Urea-PAGE-based cleavage assays we show Mn^{2+}-dependent site-specific cleavage of FL-TraI and the relaxase domain within the *oriT*. We determined the nicking-site within the *oriT* and found several additional nucleotides that are required for cleavage. Further, we set out to identify the aminoacids involved in the cleavage reaction. We generated several relaxase domain mutants with aminoacid substitutions in three conserved tyrosines and the conserved HHH- and HD-motif. We determined the catalytic tyrosine and could show, that unlike many other relaxases, no covalent intermediate is formed with the DNA. Further we show the influence of the metal-binding motifs on the cleavage reaction. This is supported by data from differential scanning fluorimetry which shows an increase of the melting temperature upon binding to Mn^{2+} ions. Additionally, high-resolution data from small-angle X-ray scattering (SAXS) of the relaxase domain shows where the *oriT*-DNA binds within the TraI structure.
Conclusion: Here, we present the first biochemical characterization of a MOB_H-family relaxase.
References
[1] W. Salgado-Pabón, S. Jain, N. Turner, C. van der Does, and J. P. Dillard, *Mol. Microbiol.*, vol. 66, no. 4, Nov. 2007.

Presentation: Monday, 6 March 2017 from 9:45 - 10:00 in room 10-11.

025/MPV
The role of the phosphodiesterase NbdA in NO-induced biofilm dispersal of *Pseudomonas aeruginosa*
M. Rüger*[1], K. Gerbracht[1], S. Zehner[1], N. Frankenberg-Dinkel[1]
[1]*TU Kaiserslautern, Microbiology, Kaiserslautern, Germany*

Pseudomonas aeruginosa is an important opportunistic human pathogen causing a variety of nosocomial infections including pneumonia, sepsis, catheter and urinary tract infections. The bacterium has become a model system for biofilm research because of its resistance to conventional antibiotics, host antimicrobial effector mechanisms and its ability to form biofilms. Dispersal is the last step of the biofilm life cycle and a process used by bacteria to transfer from sessile to motile lifestyle. Changes in c-di-GMP (bis-(3'-5')-cyclic dimeric guanosine monophosphate) levels are linked to biofilm dispersal in a number of different bacteria. The signalling molecule nitric oxide (NO) induces biofilm detachment through stimulation of c-di-GMP degrading phosphodiesterase (PDE) activity. We characterised the membrane-bound proteins MucR and NbdA (NO-induced biofilm dispersion locus A) regarding their role in NO-induced dispersal. Both proteins share an identical domain organisation consisting of the motif MHYT-GGDEF-EAL. Inactivation of *mucR* impaired biofilm detachment in response to glutamate and NO while deletion of *nbdA* only negatively affected biofilm dispersal upon exposure to NO. Biochemical analyses of recombinant protein variants lacking the membrane-anchored MHYT-domain revealed NbdA being an active PDE. In contrast, MucR showed diguanylate cyclase and PDE activity *in vitro* [1]. Interestingly, a *P. aeruginosa* strain lacking both, *nbdA* and *mucR* showed enhanced biofilm formation under tested conditions, whereas Δ*nbdA* and Δ*mucR* single mutants displayed wild type like phenotypes. The hyper biofilm formation phenotype of the Δ*nbdA* Δ*mucR* mutant might be due to highly increased intracellular c-di-GMP levels caused by lacking PDE activity of NbdA and MucR. These results suggest a possible interdependence of both proteins.

References
[1] Li Y, Heine S, Entian M, Sauer K, Frankenberg-Dinkel N. (2013) NO-induced biofilm dispersion in *Pseudomonas aeruginosa* is mediated by an MHYT domain-coupled phosphodiesterase. *J Bacteriol*. 195: 3531-3542.

Presentation: Monday, 6 March 2017 from 8:30 - 8:45 in room 5.

WORKSHOP 05
Bacterial surface structures and adhesion (FG MP)
06 March 2017 • 08:30 – 10:00

026/MPV

Primal Effects of Quorum Sensing on *Burkholderia pseudomallei* Pathogenicity

K. Stoll-Ziegler*[1], C. Kohler[2], D. Zühlke[1], L. Ziesche[3], S. Schulz[3], I. Steinmetz[4], K. Riedel[1]
[1]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*
[2]*University Medicine Greifswald, Friedrich Loeffler Institute of Medical Microbiology, Greifswald, Germany*
[3]*TU Braunschweig, Institute of Organic Chemistry, Braunschweig, Germany*
[4]*Medical University of Graz, Institute of Hygiene, Microbiology and Environmental Medicine, Graz, Austria*

Introduction: *Burkholderia pseudomallei*, the causative agent of melioidosis, is a Gram-negative soil bacterium in tropical areas. *B. pseudomallei* employs several acyl-homoserine lactone (AHL)-mediated quorum sensing (QS) systems, which regulate specific sets of genes as a function of cell density [1,2]. The genome of *B. pseudomallei* encodes genes for three QS systems with one *luxI* and one *luxR* homologue, respectively, and additionally three orphan *luxR* homologues [3]. The *luxI* homologues encode AHL synthases that produce specific AHL-molecules, which in turn bind to the respective transcriptional regulator and thereby also regulate the expression of specific genes that might be involved in virulence [4].
Objectives: The here presented study aims on dissecting the effect of the different QS-systems on *B. pseudomallei* gene expression and pathogenicity.
Materials & methods: Single and double deletion mutants were constructed and subproteomic fractions of wild-type (WT) and mutant strains were subjected to mass spectrometry analysis to identify targets of the different QS systems. For the analysis of cytosolic and extracellular proteins a DIA IMS^E approach in combination with Hi3 quantification and a GeLC MS/MS approach were applied, respectively. Moreover, the chemical nature of the AHL-molecules, synthesized by the different QS systems was determined by mass spectrometry.
Results & conclusion: Our comparative proteome analysis of WT and QS-mutants identified a significant number of differentially expressed proteins, e.g. involved in Pseudomonas quinolone signal production, polyketide and non-ribosomal peptide synthesis and type III secretion. Some of these proteins have already been described as QS-regulated in other Gram-negative opportunistic pathogens, e.g. *Burkholderia cenocepacia* [5] or *B. thailandensis* [6], and are known to be involved in pathogenicity. We are thus currently testing the pathogenic potential of single and double QS-mutants in cell-cultures and a murine pathogenicity model.

References

[1] E. Valade, F. M. Thibault and Y. P. Gauthier, J Bacteriol, Vol. 186 (2004), p. 2288.
[2] C. Majerczykin, L. Kinman and T. Han, Infect Immun, Vol. 81 (2013), p. 1471.
[3] L. Eberl, J Med Microbiol, Vol. 296 (2006), p. 107.
[4] R. L. Ulrich, D. DeShazer and E. E. Brueggemann, J Med Microbiol, Vol. 53 (2004), p. 1053f.
[5] S. Inhülsen, C. Aguilar, N. Schmid, A. Suppiger, K. Riedel, L. Eberl, MicrobiologyOpen, Vol. 1 (2012), p. 227.
[6] B. A. Duerkop, J. Varga, J. R. Chandler, S. B. Peterson, et al., J Bacteriol, Vol. 191 (2009), p. 3913.

Presentation: Monday, 6 March 2017 from 8:45 - 9:00 in room 5.

027/MPV

Identification of proteins affecting secretion of the carbohydrate binding lectin LecB of Pseudomonas aeruginosa

T. Schwabroch*[1], F. Kovacic[1], K. E. Jaeger[1]
[1]*Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Jülich, Germany*

Pseudomonas aeruginosa is a Gram-negative opportunistic human pathogen associated with chronic airway infections. Biofilms formed by *P. aeruginosa* contribute to its high resistance against antibiotics thus preventing efficient treatment of infections. Essential factors for biofilm formation and adhesion to human tissues are *P. aeruginosa* lectins LecA and LecB that bind cell-surface exposed polysaccharides[1,2]. Hence, *P. aeruginosa* lectins may serve as potential therapeutic targets. The lectin LecB of *P. aeruginosa* is a carbohydrate-binding protein attached to the cell-surface where it presumably interacts with the major porin protein OprF[3,4]. Consequently, a *P. aeruginosa* strain lacking the *oprF* gene secrets LecB into the culture medium. The mechanism of LecB secretion to the outer membrane remains to be elucidated.
To study the secretion of LecB we have screened a transposon mutant library of the *P. aeruginosa* Δ*lecB*Δ*oprF* strain carrying the p*lecB* expression plasmid for targeting LecB in the culture supernatant. For a screening, we have established a modified high-throughput enzyme-linked lectin assay (mELLA), which allows quantification of extracellular LecB. Analysis of more than 3,000 randomly selected clones revealed 47 mutants which secreted less than 15% of LecB compared to the *P. aeruginosa* Δ*lecB*Δ*oprF*/p*lecB* reference strain. To this end, we have identified several genes possibly involved in secretion of LecB. Two of them, *fgtA* and *pilY1* are involved in biogenesis of the flagellum and type-IV pili, respectively. These cellular structures are related to the adhesion and motility of *P. aeruginosa* known to be important in initial stages of the biofilm formation. Presently, we are testing a proposed model of LecB-flagellin co-secretion *via* a type-III secretion system.

References

[1] Tielker, D. *et al.* (2005) *Microbiology* 151, 1313-1323.
[2] Beuth, J. *et al.* (1987) *Eur J Clin Microbiol Infect Dis* 6, 591-593.
[3] Bartels, K. M. *et al.* (2011) *J Bacteriol* 193, 1107-1113.
[4] Funken, H. *et al.* (2012) *PLoS One* **7**, e46857.

Presentation: Monday, 6 March 2017 from 9:00 - 9:15 in room 5.

028/MPV

Loss of cortactin facilitates the invasion of intestinal epithelial cells by *Salmonella enterica* sv. Typhimurium

A. Felipe-López*[1], T. Estrada-Garcia[1], M. Hensel[2], T. Stradal[3], K. Rottner[4], M. Schnoor[1]
[1]*CINVESTAV, IPN, Molecular Biomedicine, Mexico City, Mexico*
[2]*FB05 Biologie/Chemie Universität Osnabrück, Abt. Mikrobiologie, Osnabrück, Germany*
[3]*HZI für Infektionsforschung, Zellbiologie, Braunschweig, Germany*
[4]*Zoologisches Institut - TU Braunschweig, Molekulare Zellbiologie, Braunschweig, Germany*

During invasion of *Salmonella enterica* sv. Typhimurium, enterocytes lose microvilli and suffer from strong disorganization of the F-actin cytoskeleton and, as a consequence, damage of the epithelial barrier. The F-actin binding protein cortactin supports the epithelial barrier function of the intestine by controlling actin dynamics at *tight* and *adherens junctions*. Unsworth *et al.* previously demonstrated that the recruitment of cortactin to the invasion site of *Salmonella* in epithelioid cells coincided with ruffle formation. Here, we set to investigate the role of cortactin for epithelial barrier functions during *Salmonella* invasion.
Caco-2 C2BBe1 cells were transduced using a lentivirus to downregulate the expression of cortactin. Microscopy analysis

revealed that cortactin-depleted C2BBe1 cells appeared bigger having enlarged nuclei. Furthermore, they did not establish strong epithelial barriers compared to cells expressing a scrambled shRNA (scr) or non-transduced cells. To evaluate the contribution of cortactin to *Salmonella* invasion, we infected cortactin-depleted C2BBe1 cells with the *Salmonella* WT-strain SL1344 and a non-invasive Δ*invG*-strain. The *Salmonella* WT-strain invaded cortactin-depleted C2BBe1 cells thrice more efficiently than the control C2BBe1-cells (scr or non-transducted), whereas the Δ*invG* strain infected neither the control nor cortactin-depleted cells. Confocal microscopy revealed retracted cells and severely damaged tight junctions in the absence of cortactin compared to control cells. Additionally, *Salmonella* ruffles were two times larger without cortactin. To examine the functions of cortactin *in vivo*, we infected streptomycin pre-treated WT C57BL/6 and *cortactin*$^{-/-}$ (*cttn*$^{-/-}$) mice with SL1344 and Δ*invG* strain for 48 h. SL1344 infected *cttn*$^{-/-}$ mice severely as manifested by strong weight loss, reduced intestine size, absence of faeces and reduced overall motility. Histopathological analysis of whole colon Swiss roles revealed stronger crypt erosions and increased extravasation of leukocytes in the absence of cortactin.
Therefore, we conclude that cortactin plays an important role in the restriction of *Salmonella* invasion and the maintenance of the epithelial barrier during infection. Currently, we are investigating which *Salmonella* effector protein or virulence mechanism may target cortactin and permit *Salmonella* to overcome the inhibitory functions of cortactin in this context.

Presentation: Monday, 6 March 2017 from 9:15 - 9:30 in room 5.

029/MPV

Sensitizing *Staphylococcus aureus* to cationic antimicrobial peptides by MprF-inhibiting monoclonal antibodies

C. Slavetinsky*[1,2], C. M. Ernst[1,2], A. Kraus[3], A. Geyer[1,2], A. Peschel[1,2]
[1]*Interfaculty Institute of Microbiology and Infection Medicine, University of Tübingen, Infection Biology, Tuebingen, Germany*
[2]*German Center for Infection Research, Tübingen, Germany*
[3]*Morphosys AG, Martinsried, Germany*

The lysinylation of negatively charged phosphatidylglycerol by MprF (the Multiple peptide resistance Factor) confers resistance to cationic antimicrobial peptides (CAMPs), several antibiotics (e.g. daptomycin) and the host immune system in general (1). MprF of *Staphylococcus aureus* is a bifunctional enzyme consisting of separable domains for lysyl phosphatidylglycerol (LysPG) production and LysPG flipping (2).
As MprF proteins are crucial virulence factors for various bacterial pathogens and play a major role in the emerging resistance against the blockbuster antibiotic daptomycin, they represent an attractive target for novel anti-virulence drugs. We designed several monoclonal antibodies in order to block MprF and analyzed their capacity to support the killing of *S. aureus* by CAMPs, antibiotics, and by human neutrophils. We found that those antibodies block the flippase reaction of MprF, which (i) uncovers its enzymatic center showing dynamic topology changes during flipping and (ii) renders *S. aureus* more susceptible to clearance by the host immune system and antibiotics. Most notably they restore the susceptibility of a daptomycin-resistant clinical isolate, prevent growth of a CA-MRSA strain under subinhibitory concentrations of antibiotics and protect mice from severe infection in a skin abscess model.
These findings provide a promising new approach for anti-virulence therapy against bacteria based on pathogen sensitization to host immune system or antibiotics. Furthermore, they offer interesting options for studying the MprF mode of action and its role in daptomycin resistance.

References

[1] Peschel A, Jack RW, Otto M, Collins LV, Staubitz P, Nicholson G, Kalbacher H, Nieuwenhuizen WF, Jung G, Tarkowski A, van Kessel KP, van Strijp JA. The Journal of experimental medicine. 2001. 193**:**1067-1076.
[2] Ernst CM, Staubitz P, Mishra NN, Yang SJ, Hornig G, Kalbacher H, Bayer AS, Kraus D, Peschel A. PLoS Pathog. 2009. 5**:**e1000660.

Presentation: Monday, 6 March 2017 from 9:30 - 9:45 in room 5.

030/MPV

A novel gonorrhea vaccine candidate, NGO1985, is a surface-exposed lipoprotein involved in cell envelope homeostasis via interaction with the Bam complex

A. Sikora*[1], I. Wierzbicki[1], R. Zielke[1], A. Begum[2], M. Unemo[3], A. Jerse[2]
[1]*Oregon State University, College of Pharmacy , Corvallis, United States*
[2]*F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, Department of Microbiology and Immunology, Washington, United States*
[3]*Örebro University Hospital, Department of Laboratory Medicine, Microbiology, Örebro, Sweden*

Neisseria gonorrhoeae (GC) rapidly acquires antibiotic resistance, which seriously compromises the arsenal of available treatment for patients with gonorrhea. This sexually transmitted infection remains a significant burden on reproductive and neonatal health worldwide, making the development of gonorrhea vaccine the highest priority. To identify potential gonococcal vaccine antigens, we applied a proteomics-driven antigen mining of cell envelopes and naturally released membrane vesicles (MVs) derived from four common laboratory GC strains FA1090, MS11, F62, and 1291. NGO1985 was identified as one of the ubiquitously expressed proteins localized to the cell envelopes and membraned vesicles in analyzed GC isolates. Initial characterization of this predicted outer membrane lipoprotein showed that loss of NGO1985 resulted in a dramatic decrease in gonococci viability when exposed to a variety of chemical probes. Here we demonstrated that in addition to function in the maintenance of GC cell envelope integrity, NGO1985 is a promising gonorrhea vaccine candidate. Corroborating our initial observations, deletion of NGO1985 in a panel of recent GC clinical isolates restored their susceptibility to multiple antibiotics as assessed by E-tests. Further, the compromised integrity of the cell envelope in gonococci lacking NGO1985 was evidenced by significant increase in soluble protein content and overall amount of MVs, as well as dramatically altered protein profiles of cell envelopes and MVs in comparison to that of the wild type FA1090 strain. To elucidate the function of NGO1985 in the cell envelope homeostasis, interactome profiling involving pull-down experiments coupled with proteomics were undertaken. These studies showed that NGO1985 interacts with β-Barrel Assembly Machinery (Bam) complex, antibiotic efflux pump(s) including Mtr, and several known, as well as previously uncharacterized lipoproteins. The examination of fitness of gonococci lacking NGO1985 revealed that this protein significantly contributes to survival in the presence of normal human serum, anoxia, and in the mouse genital tract. We also present evidence that NGO1985 is a surface-exposed lipoprotein using a variety of approaches including a site-directed mutagenesis of conserved cysteine within the predicted lipobox motif, immunodots and immunogold labeling of intact gonococci, and protease accessibility studies. Finally, NGO1985 is highly conserved and expressed in a panel of geographically and temporally diverse GC strains.
We conclude that NGO1985 is a promising gonorrhea vaccine candidate due to its pivotal function in the gonococci cell envelope integrity, surface localization, ubiquitous expression and high-degree of conservation among diverse GC isolates.

Presentation: Monday, 6 March 2017 from 9:45 - 10:00 in room 5.

MINISYMPOSIA 06
Food Microbiology and Food Hygiene (FG LM)
06 March 2017 • 08:30 – 10:00

031/LMV

Listeriosis in Germany 2010-2016, trends and clusters

H. Wilking*[1], S. Halbedel[2], S. Kleta[3], W. Ruppitsch[4], S. Huhulescu[4], L. Bös[1], F. Allerberger[4], K. Stark[1], R. Prager[2], A. Flieger[2]
[1]*Robert Koch Institute (RKI), Division for Gastrointestinal Infections, Zoonoses and Tropical Infections, Berlin, Germany*
[2]*Robert Koch Institute (RKI), Division Enteropathogenic Bacteria and Legionella, Wernigerode, Germany*
[3]*Federal Institute for Risk assessment (BfR), National Reference Laboratory for Listeria, Berlin, Germany*
[4]*Austrian Agency for Health and Food Safety (AGES), German-Austrian Binational Consiliary Laboratory for Listeria, Vienna, Austria*

Listeriosis is a mandatory notifiable foodborne infectious disease. Especially older adults, pregnant women, and immunocompromised persons are at risk for disease, which presents as septicemia, central nervous system infection or fetal infection.
We analyzed national surveillance data from 2010 to 2016 and described the distribution of invasive listeriosis in Germany. Non-invasive cases were excluded and pregnancy associated listeriosis was defined as one case. Time trends, geographical distributions and risk groups were reported. Special focus is placed on systematic typing of patients" isolates in two reference centers in Wernigerode and Vienna and the detection of a large outbreak with molecular tools.
In total 3,337 cases were recorded during the study period of 7 years, thereof 165/3,337 (5%) pregnancy-associated. Annual case numbers increased from 343 in 2010 to 653 in 2016. Among 3,172 non-pregnancy-associated cases 1,277 (40%) were female and 2,189 were aged above 64 years. Fetal loss was reported for 41/165 (25%) pregnancy-associated cases and death in 348/3,172 (11%) resulting in an overall case-fatality of 12%. Clinical samples included blood in 2,550/3,172 (80%), cerebrospinal fluid in 422/3,172 (13%) and from other usually sterile sites in 177/3,172 (6%). From 2013 to 2016 for 36% of the notified cases a typing result could be retrieved. The largest cluster was an outbreak from 2012 to 2016 with 78 cases in southern Germany. NGS revealed the unique cluster type CT1248. Systematic typing of food isolates identified a meat producing facility supplying supra-regional supermarket grocery chains as the source of infection.
Listeriosis is the notifiable infectious disease with the highest case fatality and an increasing disease burden is observed. Systematic typing of clinical *Listeria monocytogenes* isolates leads to the detection of clusters, to the successive investigation of related outbreaks and leads to the control of contamination in food vehicles. This might have the greatest impact on reducing the burden of listeriosis. Therefore all stakeholders (clinicians, diagnostic laboratories and public health offices) are requested to send any *Listeria monocytogenes* clinical isolate to one of the reference centers in Wernigerode or Vienna to increase the proportion of cases with molecular information.

Presentation: Monday, 6 March 2017 from 8:30 - 9:00 in room 12.

032/LMV

***Arcobacter butzleri*, an emerging food pathogen and its susceptibility profile**

S. Zimmermann*[1], I. Burckhardt[1], A. Dalpke[1], Y. Uehara[2], Y. Yamauchi[1]
[1]*Zentrum für Infektiologie UKHD, Institut für Med. Mikrobiologie, Heidelberg, Germany*
[2]*Juntendo University, 2. Department of Infection Control Science, Tokyo, Japan*

Introduction: Arcobacter spp. was more recently identified in food animals and variety of foods including seafood. In human the pathogen causes most commonly diarrhea.
Objectives: This study tried to determine the frequency of this emerging enteric pathogen in fecal samples of patients from a university hospital. In addition the isolated *Arcobacter butzleri* strains were typed on a species level and the minimum inhibitory concentration (MIC) distribution data were determined to evaluate susceptibility to antibiotics commonly used for diarrheal illness.
Patients and Methods: 1000 fecal samples from patients were collected between January and September 2016 and were analysed by a genus specific real time-PCR for the presence of Arcobacter DNA. 40 *Arcobacter butzleri* strains were isolated from patients and MIC values were determined using gradient strip method (E-test). EUCAST breakpoints for *Campylobacter spp.* was used to evaluate antibiotic susceptibility to ciprofloxacin, erythromycin and doxycycline.
Results: 6 out of 1000 stool samples were PCR positive for Arcobacter (5 *A. butzleri* and one *A. skirrowii*). 48 Campylobacter isolates were detected in our samples in the same period. More than 10% of enteric pathogens isolated from campylobacter selective medium were *Arcobacter spp.*
The incidence of Arcobacter in stool samples over all was lower than 1%, but within the detected Campylobacteraceae the percentage was more than 10%. Therefore the emerging pathogen was found more frequently than the published range of 0,4-4%.
72.7% of *Arcobacter butzleri* strains were susceptible to ciprofloxacin. Doxycycline retained moderate activity against *Arcobacter butzleri* (63.6%). Only 39.4% *Arcobacter butzleri* strains were susceptible to erythromycin.
Conclusions: Most *Arcobacter butzleri* were susceptible to ciprofloxacin. Resistance to erythromycin was high and susceptibility to doxycycline was moderate. Fluoroquinolones can be suggested for treatment of *Arcobacter butzleri* related gastrointestinal infections, but antibiotic susceptibility testing is recommended to prevent treatment failures. Further investigations should elucidate resistance mechanisms and genes of this recently described enteric pathogen.

Presentation: Monday, 6 March 2017 from 9:00 - 9:15 in room 12.

033/LMV

Food Bacteria Interplay: Concerted action of extrinsic and extrinsic factors gearing toxin synthesis in emetic *Bacillus cereus*

M. Ehling-Schulz*[1], M. Kranzler[1], G. Lücking[2], S. Marxen[2], K. Rouzeau-Szynalski[3], M. Sulyok[4], T. Stark[2], E. Frenzel[1,5]
[1]*Vetmeduni Vienna, Institute of Microbiology, Pathobiology, Vienna, Austria*
[2]*Technische Universität München, Freising, Germany*
[3]*Nestec Ltd, Nestlé Research Center, Lausanne, Switzerland*
[4]*University of Natural Resources and Life Sciences, Vienna, Austria*
[5]*University of Groningen, Groningen, Netherlands*

The incidence of foodborne intoxications caused by bacterial toxins is steadily increasing worldwide throughout the last decade. Especially the toxin cereulide, produced by emetic *Bacillus cereus*, is increasingly recognized as a serious threat implicated in severe clinical manifestations, such as acute liver failure. Cereulide is a dodecadepsipeptide that is produced by an unusual non-ribosomal peptide synthetase, named cereulide synthetase (Ces NRPS). We recently showed that the Ces NRPS, which does not follow the canonical NRPS biosynthetic logic for peptide assembly but represents a novel biosynthesis mechanism of ester bond containing NRPS products, generates a huge diversity of

chemical cereulide toxin variants with highly variable toxigenic potential.
Cereulide synthesis is controlled by complex and tightly regulated networks that are inextricably linking toxin gene expression to life cycle phases and specific conditions, such as the nutrient supply encountered in food matrices. Considerable progress has been made in the molecular and biochemical characterization of cereulide toxin synthesis while information about extrinsic signals acting on toxin production in the food environment is still limited [1].
We therefore investigated the influence of temperature, which is an important extrinsic parameter in food production and processing, on cereulide synthesis. Our systematic approach revealed temperature as a cardinal environmental effector of post-transcriptional cereulide synthesis regulation. Temperature does not only significantly influence the toxin production levels but also alters the composition of cereulide isoforms. While bacterial intrinsic factors exert intensive control of cereulide synthesis on a transcriptional level, external factors, such as temperature, influence cereulide synthesis primarily on a posttranscriptional level. How internal and external signals are integrated to link toxin synthesis to the bacterial metabolism and life cycle is subject of ongoing research. We will discuss the role of intrinsic and extrinsic factors acting on cereulide toxin biosynthesis and stress how unravelling these processes can open new avenues for the development of effective strategies to prevent toxin synthesis in food production and processing.

References
[1] Ehling-Schulz et al., Frontiers in Microbiology 2015

Presentation: Monday, 6 March 2017 from 9:15 - 9:30 in room 12.

034/LMV
FTIR metabolomic fingerprint reveals different modes of action exerted by active pharmaceutical ingredient based ionic liquids (API-ILs) on *Salmonella* Typhimurium
P. Mester*[1], A. K. Witte[1], C. Leeb[1], R. Kalb[2], T. Grunert[3], P. Rossmanith[1]
[1]*Christian Doppler Laboratory for Monitoring of Microbial Contaminants, Vienna, Austria*
[2]*Proionic GmbH, Graz, Austria*
[3]*University of Veterinary Medicine, Institute of Microbiology,, Vienna, Austria*

Introduction: Since their incorporation into various chemical and biochemical processes, ionic liquids (ILs) have now been found useful for biomedical applications, including active pharmaceutical ingredients (APIs) such as antimicrobial agents or antibiotics. Recently, synergistic API-ILs with great potential have been reported, which show either increased antimicrobial activity or the ability to overcome bacterial resistance.
Objectives: In this study a total of 19 API-ILs, based on the antibiotic nalidixic acid, combined with different cation species, as well as 19 respective chloride ILs were investigated for synergistic effects against the important foodborne pathogen *Salmonella*.
Materials and Methods: The antimicrobial activities of all 38 ILs against six different *Salmonella* species, as well as two nalidixic acid-resistant *S.* Typhimurium strains, were determined via the microbroth dilution assay. The response pattern of the main cellular constituents, namely proteins, carbohydrates, and lipids of the bacterial cells to the most promising API-ILs was further investigated by Fourier transform infrared (FTIR) spectroscopy.
Results: While a number of active API-ILs based on nalidixic acid could be synthesized, no evidence for synergistic effects, such as increased antimicrobial activity or the ability to overcome resistance was found with either microbiological or spectroscopic methods. In the case of API-ILs with non-toxic cations, the activity of nalidixate as an antibiotic remained unchanged, both against susceptible and resistant strains. FTIR spectroscopic analysis of two IL species with similar high antimicrobial activity ([TC8MA] and [TMC16A]) did not distinguish between the anions (nalidixate and chloride) but differentiated clearly between the cations, indicating alterations in the bacterial membrane Fluidity. Thus, FTIR spectroscopy revealed different modes of action of differentially modified ILs with similar MICs against *S.* Typhimurium.
Conclusion: Microbiological as well as spectroscopic methods revealed that, in the case of the foodborne pathogen Salmonella, no synergistic effects of the API-ILs could be found. The present study demonstrates that FTIR spectroscopy is a very informative, high-throughput method to determine IL-induced changes in bacteria on macromolar and metabolic levels. It is anticipated that FTIR spectroscopy, followed by chemometrics, is also suitable for developing statistical models capable of predicting changes in membrane Fluidity or other cellular biochemical constituents associated with ILs.

Presentation: Monday, 6 March 2017 from 9:30 - 9:45 in room 12.

035/LMV
Biochemical fate of vicilin storage protein during fermentation and drying of cocoa beans
N. Kumari*[1], W. John[1], N. Kuhnert[1], M. Ullrich[1]
[1]*Jacobs University Bremen, Life Sciences and Chemistry, Bremen, Germany*

Key cocoa-specific aroma precursors are generated during the fermentation of cocoa beans via the proteolysis of the vicilin-like globulin. Previous studies had shown that degradation of this particular 566 amino acid-long storage protein leads to three distinct subunits with different molecular masses. Although oligopeptides generated from the proteolysis of vicilin-like globulin have been studied previously, changes occurring to vicilin at different stages of fermentation have not yet been explored in detail. The aim of this study was to investigate the fate of vicilin protein from the non-fermented stage up to the dried cocoa beans and to perform a comparative analysis of the peptides generated from the degradation of vicilin protein in a commercial fermentation with that of the artificial fermentation system, free from microbial activity. The protein profile was analyzed using SDS-PAGE and 2D-PAGE analytical methods. All major protein spots obtained were subjected to proteolytic digestion and MALDI-MS analysis in order to assign them to respective cocoa proteins. Our results showed a remarkable shift in the electrophoretic mobility of vicilin towards higher pI during the onset of fermentation. The pI-shifted subunit was found susceptible to further degradation into a lower-molecular-weight vicilin subunit. The observed pI shift correlated with, but did not depend on protein phosphorylation. Glycosylation of some but not all vicilin subunits occurred at different stages of the fermentation process. Peptides generated from vicilin throughout fermentation (commercial as well as artificial) were analyzed by UHPLC-ESI-MS/MS. Peptides generated from the two different fermentation methods showed nearly ninety percent similarity and revealed an initial increase and subsequent decrease in their diversity with an increasing degree of fermentation. We furthermore describe the rate of degradation of different vicilin subunits. The detected diversity and dynamics of vicilin peptides will help to define biochemical markers of distinct steps of the fermentation process.

Presentation: Monday, 6 March 2017 from 9:45 - 10:00 in room 12.

WORKSHOP 07
Humans, Animals and Resistance (StAG KM)
06 March 2017 • 08:30 – 10:00

036/KMV

Impact of the colistin resistance gene *mcr-1* on bacterial fitness

M. Tietgen[1], T. Semmler[2], V. A. J. Kempf[1], S. Riedel-Christ[1], A. Hamprecht[3], C. Ewers[4], S. Göttig*[1]
[1]*Goethe University Hospital Frankfurt, Institute of Medical Microbiology and Infection Control, Frankfurt, Germany*
[2]*Robert Koch Institute, Berlin, Germany*
[3]*University Hospital of Cologne, Institute of Medical Microbiology, Immunology and Hygiene, Cologne, Germany*
[4]*Justus-Liebig-University Giessen, Institute of Hygiene and Infectious Diseases of Animals, Giessen, Germany*

Introduction: Colistin (polymyxin E, CT) is an important last-resort antibiotic used in the treatment of infections with multidrug resistant Gram-negative pathogens. Since the discovery of the first plasmid-borne colistin resistance determinant *mcr-1* in *Escherichia coli* in 2015, the rapid dissemination of CT-resistant bacteria has to be expected.
Objectives: The objective was to determine the impact of the CT resistance gene *mcr-1* on fitness of *E. coli* and *Klebsiella pneumoniae* since acquisition of resistance determinants often results in a loss of fitness.
Materials & methods: Horizontal gene transfer of *mcr-1* carrying plasmid pKP2442 was evaluated by liquid mating employing sodium azide-resistant *E. coli* J53 and *K. pneumoniae* PRZ as recipients. Plasmid analysis was done by next generation sequencing (Illumina, MiSeq). Non-competitive and competitive growth kinetics were analyzed using LB and M9 minimal medium. Cytotoxicity to human cells was assessed *in vitro* using A549 human lung epithelial cells via the lactate dehydrogenase (LDH) assay. *In vivo* virulence was investigated using larvae of the Greater wax moth (*Galleria mellonella*).
Results: A *Klebsiella pneumoniae* of sequence type ST2299, harbouring a 217 kb IncHI2-type plasmid termed pKP2442 was isolated from a leukaemia patient. pKP2442 could be mobilized by intra- and intergenus transconjugation from the clinical isolate to recipient strains *E. coli* J53 (transconjugation frequency: 6.9 x 10-8 ± 5.6 x 10-8) and *K. pneumoniae* PRZ (4.0 x 10-8 ± 3.0 x 10-8), respectively. After infection of A549 lung cells with pKP2442 transconjugants and their respective parental strains equal LDH activities were measured indicating no significant impact of *mcr-1*. Survival of *G. mellonella* larvae infected with *mcr-1* expressing strains and controls was similar for *E. coli* J53 and *K. pneumoniae* PRZ. Growth experiments showed no differences in doubling times for both *E. coli* pKP2442 and *K. pneumoniae* pKP2442 compared to their parental strains. Competitive growth experiments also revealed no significant changes in growth rates between *E. coli* J53 pKP2442 transconjugants and their parental strain (selection rate constant: 0.01 ± 0.29). In contrast, *K. pneumoniae* PRZ pKP2442 transconjugants showed significantly reduced growth rates compared to their parental strain (selection rate constant: -1.62 ± 0.49) indicating a decrease in fitness.
Conclusions: The data indicate that acquisition of plasmid-borne *mcr*-1 does not impair fitness in *E. coli* J53 but negatively influences growth rates in *K. pneumoniae* PRZ. This might at least partially explain why *mcr-1* is present almost exclusively in *E. coli* but less abundant in other species like *K. pneumoniae*. The efficient transfer of the *mcr-1* containing plasmid in human pathogenic isolates which does not affect bacterial fitness suggests the further dissemination of colistin-resistant Gram-negative pathogens.

Presentation: Monday, 6 March 2017 from 8:30 - 8:45 in room 7-8.

037/KMV

Wound infections in patients from a rural setting in Ghana – bacterial spectrum and antimicrobial susceptibility

H. O. Janssen[1], I. Janssen[2], P. Cooper[3], C. Kainyah[3], T. Pellio[3], M. Quintel[1], U. Groß[2], M. H. Schulze*[2]
[1]*University Medical Center Göttingen, Department of Anaesthesiology, Göttingen, Germany*
[2]*University Medical Center Göttingen, Institute for Medical Microbiology and Göttingen International Health Network, Göttingen, Germany*
[3]*St. Martin de Porres Hospital, Eikwe, Ghana*

Question: Wound infections after injuries during daily life activities, e.g. farming, or post-surgery are common medical problems in the tropics. Almost always antibiotic treatment is given to the patient without knowing the causing bacterial pathogens and their antibiotic susceptibility as no facility for bacteriological investigation is available or the patient has no money to pay for. Often, this leads to multiple antibiotic courses without clinical improvement. It also contributes to further increase antimicrobial resistance.
Methods: During a four-month period wound swabs from 67 patients taken from: lower extremity - 38 patients; upper extremity - 4 patients; trunk - 16 patients; and laparotomy wounds - 9 patients, and bacteriological investigation and susceptibility testing were performed.
Results: Overall, 32 different bacterial species were isolated from the 67 specimens. Between one and seven different bacterial species were detected in a single specimen. Wound swabs from the lower extremity were found to contain the highest number of different bacteria. Most frequently were found: *Staphylococcus (S.) aureus* in 31 (46%), *Enterococcus faecalis* in 24 (36%), *Pseudomonas aeruginosa* in 20 (30%), *Proteus mirabilis* in 20 (30%), *Escherichia* (*E.*) *coli* in 19 (28%), *Klebsiella (K.) pneumoniae* 13 (19%), *Enterobacter (E.) cloacae* complex in 10 (15%) and *Acinetobacter baumannii* complex in 8 (12%) of 67 wound swabs.
All *S. aureus*-isolates were Oxacillin susceptible.
The susceptibility of the *E. coli*-isolates were: 8 isolates (42%) susceptible to 3rd Generation Cephalosporines (3rd Gen. Ceph.) and to Ciprofloxacin (CIP); 2 isolates (11%) susceptible to 3rd Gen. Ceph. and resistant to CIP; 1 isolate (5%) resistant to 3rd Gen. Ceph. and susceptible to CIP; and 8 isolates (42%) resistant to 3rd Gen. Ceph. and to CIP. No Carbapenem resistance was detected.
The susceptibility of the *K. pneumoniae*-isolates were: 7 isolates (54%) susceptible to 3rd Gen. Ceph. and to CIP; and 6 isolates (46%) resistant to 3rd Gen. Ceph. and to CIP. No Carbapenem resistance was detected.
The susceptibility of the *E. cloacae* complex-isolates were: 6 isolates (60%) susceptible to 3rd Gen. Ceph. and to CIP; 1 isolate (10%) resistant to 3rd Gen. Ceph. and susceptible to CIP; and 3 isolates (30%) resistant to 3rd Gen. Ceph. and to CIP. No Carbapenem resistance was detected.
Conclusion: Globally increasing antimicrobial resistance especially in gram-negative bacteria requires bacteriological investigation and susceptibility testing to avoid ineffective antibiotic treatment and wasting of resources. Even in regions with limited resources, like in Sub-Saharan Africa, basic bacteriology including resistance testing is possible.

Presentation: Monday, 6 March 2017 from 8:45 - 9:00 in room 7-8.

038/KMV

Evidence for avian reservoirs of the nosocomial pathogen *Acinetobacter baumannii*

G. Wilharm*[1], E. Skiebe[1], U. Blaschke[1], M. Poppel[1], C. Heider[1], M. Heindorf[1], S. Leser[1], C. Cuny[2], Y. Pfeifer[2], F. Layer[2], S. Fuchs[2], J. Bender[2], M. Tobółka[3], K. Żołnierowicz[3], M. Kasprzak[4], M. Bochenski[4], P. Higgins[5], H. Seifert[5], T. Semmler[6], L. Jerzak[4]
[1]*Robert Koch-Institut, P 2, Wernigerode, Germany*
[2]*Robert Koch-Institut, FG 13, Wernigerode, Germany*
[3]*Poznań University of Life Sciences, Institute of Zoology, Poznań, Poland*
[4]*University of Zielona Góra, Faculty of Biological Sciences, Zielona Góra, Poland*
[5]*Universität Köln, Institut für Medizinische Mikrobiologie, Immunologie und Hygiene, Köln, Germany*
[6]*Robert Koch-Institut, NG 1, Berlin, Germany*

The natural habitats and potential reservoirs of the nosocomial pathogen *Acinetobacter baumannii* are poorly defined. Here, we have put forth and tested the hypothesis of avian reservoirs of *A. baumannii*. We screened tracheal and rectal swab samples from livestock (chicken, geese) and wild birds (white stork nestlings) and isolated *A. baumannii* from 3% of sampled chicken (n=220), 8% of geese (n=40) and 16% of white stork nestlings (n=215). Virulence of selected avian *A. baumannii* isolates was comparable to that of clinical isolates in the *Galleria mellonella* infection model. Whole genome sequencing revealed the close relationship of an antibiotic-susceptible chicken isolate from Germany with a multidrug-resistant human clinical isolate from China. Moreover, we identified a stork isolate from Poland related to a human clinical isolate from the USA. Avian isolates do not form a distinct clade within the phylogeny of *A. baumannii*, instead they diverge into different lineages. Further, we provide evidence that *A. baumannii* is constantly present in the habitats occupied by storks. Collectively, our study suggests *A. baumannii* to be a zoonotic organism that disseminates into livestock.

Presentation: Monday, 6 March 2017 from 9:00 - 9:15 in room 7-8.

039/KMV

Adaptation driven metabolic rewiring of *Pseudomonas aeruginosa* isolated from Cystic Fibrosis patient

R. La Rosa*[1], S. Molin[1], H. Krogh Johansen[1,2]
[1]*DTU Biosustain, Lyngby, Denmark*
[2]*Rigshospitalet, Department of Clinical Microbiology, København, Denmark*

The opportunistic pathogen *P. aeruginosa,* causes chronic lung infections in cystic fibrosis (CF) patients that can last for decades. Over the course of the infection, mutations in the bacterial genomes are accumulated, leading to increased fitness relative to non-adapted strains. Although some mutations associated with successful infection have been identified and characterized, it is still unclear how mutations in regulatory and metabolic genes contribute to the infection. Metabolism represents the economy of bacteria and it is configured accordingly to the environment colonized by the bacteria. To evaluate how *P. aeruginosa* metabolism adapts to the lung environment over the course of the infection, we performed a comprehensive metabolite profiling of culture supernatants (metabolic footprinting) of strains isolated from a patient infected by three different *P. aeruginosa* clonal types at different stages in their evolution process. Lower growth rate, reduced metabolic capabilities, adaptation dependent hierarchy of assimilation of carbon sources, reduced fermentation processes and increased resistance to stresses were observed in the more adapted isolates relative to the less adapted strains. Altogether these results confirm that during long-term infection in the lungs, *P. aeruginosa* displays a reduced metabolic repertoire in addition to a more efficient use of the resources, resulting in increased fitness and robustness during persistence in the host.
Presentation: Monday, 6 March 2017 from 9:15 - 9:30 in room 7-8.

040/KMV

***Pseudomonas aeruginosa* modulates the antiviral response of airway epithelial cells**

J. Kantorek*[1,2], L. Byrnes[1], S. Tümkaya[1], M. Stahl[3,2,4], S. Graeber[1,2,4], M. Mall[1,2,4], S. Dittrich[5,2,4], S. Boutin[1,2], D. Nguyen[6], A. Dalpke[1,2], M. Weitnauer[1]
[1]*Universitätsklinikum Heidelberg, Dept. of Infectious Diseases, Heidelberg, Germany*
[2]*German Center for Lung Research (DZL), Translational Lung Research Center Heidelberg (TLRC), Heidelberg, Germany*
[3]*Universitätsklinikum Heidelberg, Division of Pulmonology & Allergology and cystic Fibrosis Center, Heidelberg, Germany*
[4]*Universitätsklinikum Heidelberg, Dept. of Translational Pulmonology, Heidelberg, Germany*
[5]*Universitätsklinikum Heidelberg, Dept. of Pneumology and Critical Care Medicine, Heidelberg, Germany*
[6]*McGill University, Dept. of Medicine , Montreal, Canada*

Objectives: Patients suffering from the genetic disease cystic fibrosis (CF) often become infected with the Gram-negative, rod-shaped bacterium *Pseudomonas aeruginosa* (*P.a.)* especially at later time points of disease. *P.aeruginosa* infection is linked to intermittently emerging pulmonary exacerbations which are the main drivers of lung function worsening. Since exacerbations in other respiratory diseases like asthma and COPD have been linked to infections with respiratory viruses we investigated the influence of *P.aeruginosa* on the antiviral host immune response.
Methods and Results: Airway epithelial cells were pretreated with conditioned medium (CM) of different *P.aeruginosa* strains and subsequently infected with Respiratory Syncytial Virus (RSV). CM of *P.aeruginosa* could significantly repress the secretion and induction of both Interferons (IFN) and antiviral proteins (MX1, OAS1) in comparison to the control, thus facilitating viral replication and spreading. Primary recognition of RSV did not seem to be altered, since initial IFN type III expression was not affected by *P.aeruginosa*. Heat treatment or filtration (MWCO 30kDa) of *P.aeruginosa* CM restored the anti-viral response. Moreover, CM of *P.aeruginosa* was able to degrade IFN type III directly, suggesting the involvement of secreted proteases. Additionally, further experiments using *P.aeruginosa* isolates of patients suffering from CF at different infection stages revealed a dependency on the Quorum-sensing system LasR which is known to be a transcriptional activator of three proteases (LasA, LasB, AprA). Previous studies indicated that *LasR* is subject to a high mutation rate causing loss of function in chronic *P. aeruginosa* infection stages. In line with this, a pilot study revealed a higher prevalence of rhinovirus in CF sputum sampled from CF patients being affected by an intermittent *P. aeruginosa* infection compared to those with a chronic course of disease.
Conclusions: In conclusion, secreted proteases by *P.aeruginosa* might promote virus induced exacerbations in cystic fibrosis patients by decreasing the antiviral response through degradation of IFN type III.

Presentation: Monday, 6 March 2017 from 9:30 - 9:45 in room 7-8.

041/KMV
The Human Lung Microbiome in Subtypes of Chronic Obstructive Pulmonary Disease (COPD)
M. Engel*[1], D. Endesfelder[1], B. Schloter-Hai[2], S. Kublik[2], L. Ziegler-Heitbrock[3], M. Schloter[2], W. zu Castell[1,4]
[1]*Helmholtz Zentrum München, Research Unit Scientific Computing, Neuherberg, Germany*
[2]*Helmholtz Zentrum München, Research Unit Environmental Genomics, Neuherberg, Germany*
[3]*Helmholtz Zentrum München, EvA Study Center, Gauting, Germany*
[4]*Technische Universität München, Department of Mathematics, München, Germany*

Question: Chronic Obstructive Pulmonary Disease (COPD) is used as collective term for a group of non-communicable lung diseases, which are characterized by chronic cough, increased sputum production and dyspnoea particularly upon exertion. Changes in microbial community composition in the lung of patients suffering from moderate to severe COPD forms have been well documented [1]. Meanwhile, based on CT, COPD subtypes have been defined: the airway dominated subtype, the emphysema dominated subtype, and cases without abnormalities in CT. We adressed the question, whether different COPD subtypes are associated with specific changes in the microbiome of the lung.
Methods: The bacterial community composition derived from brush samples from lungs of 16 patients suffering from different subtypes of COPD and 10 healthy subjects was analyzed using a cultivation independent barcoding approach applying 454-pyrosequencing of 16S rRNA gene fragment amplicons.
Results: Bacterial community composition in patients suffering from airway- and emphysema-type COPD (severe subtypes) was different from that in lungs of patients without visible changes in CT as well as from healthy subjects (mild subtype/control group) (PC1, P=0.0037).
Several genera showed significant differences in abundance between the two groups of individuals. For example *Streptococcus* (P=0.03), showed significantly higher abundance in severe COPD cases. In the mild subtype/control group significantly higher abundances of genera *Prevotella* (P=0.008), or *Parvimonas* (P=0.0038) were contributing to the observed differences.
Co-occurrence analysis suggests the presence of networks of co-occurring bacteria. Clustering of genera into communities indicated that co-occurrence relationships were strongly influenced by differences in microbial abundances between severe cases and the mild subtype /control group.
Conclusions: Detection of shifts in community composition of cases with mild to moderate COPD and detected abnormalities in CT in our study suggests that changes in microbial colonization of the lungs of COPD patients already occur early, before meaningful impairment of the health status starts. Our findings indicate that physiological differences in the lung of patients suffering from different subtypes of COPD go along with alterations in bacterial communities, which may induce further changes in the interaction between microbes and host cells. This might result in a changed interplay with the host immune system.

References
[1] Pragman AA, Kim HB, Reilly CS, Wendt C, Isaacson RE. The Lung Microbiome in Moderate and Severe Chronic Obstructive Pulmonary Disease. PLoS ONE. 2012;7(10):e47305. doi: 10.1371/journal.pone.0047305.
[2] Global Initiative for Chronic Obstructive Lung Disease G. From the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015. 2015
[3] Subramanian DR, Gupta S, Burggraf D, vom Silberberg SJ, Heimbeck I, Heiss-Neumann MS, et al. Emphysema- and airway-dominant COPD phenotypes defined by standardised quantitative computed tomography. European Respiratory Journal. 2016;48(1):92-103

Presentation: Monday, 6 March 2017 from 9:45 - 10:00 in room 7-8.

WORKSHOP 08
Epidemiology, pathogenicity and antimicrobial resistance of zoonotic pathogens (FG ZO)
06 March 2017 • 08:30 – 10:00

042/ZOV
Endemic Coxiellosis in dairy cattle herds
J. Böttcher*[1], B. Motsch[2], E. Deckinger[3], S. Werth[3], M. Alex[1], B. Janowetz[1]
[1]*Bavarian Animal Health Service, Diagnostic Department, Poing, Germany*
[2]*School of Life Science, Institut of Animal Physiology and Immunology, Freising, Germany*
[3]*Bavarian Animal Health Service, Cattle Health Service, Poing, Germany*

Introduction: Dairy cattle farms are frequently infected with the zoonotic pathogen *Coxiella burnetii* (Cb). Currently, phase-specific antibody tests are not available for ruminants.
Objective: A phase I (PhI) and phase II (PhII)-specific antibody ELISA and an IFN-γ-recall assay were established. By the application of these tests a model of endemic Cb-infection in dairy cattle farms was postulated.
Materials and Methods: Milk samples from 2429 cows (44 farms) were collected. PhI and PhII antibodies were quantitatively determined by titration in ELISA. Age-dependent immune-profiles for herds were analyzed. In order to determine changes of immune-profiles, two farms were tested longitudinally over periods of 3 (3 samplings) and 2 years (4 samplings). Finally, in a third farm blood samples of all animals were also in order analyzed for IFN-γ after stimulation with PhII-antigen.
Results: Three distinct immune-profiles we defined at herd-level as acute (14 farms, 750 samples), chronic (10 farms, 714 samples) and silent (20 farms, 963 samples including two farms which were completely negative): Acute profiles were characterized by PhII-antibodies in primiparous cows; the preceding age-group was frequently seronegative. In chronic cases PhI-antibodies were additionally detected in late first lactation and older cows. The silent stage was defined by seronegative primiparous cows, although antibodies and even Cb were detected in distinct older cows. The transition between these stages was demonstrated in two herds over time. In order to elucidate the value of antibody-negative primiparous cows as observed in the silent stage, IFN-γ-responses were assessed in one chronically infected herd. Strong IFN-γ-responses were observed in seronegative replacement heifers; and in young calves we detected IFN-γ-reactivity and phase II-antibodies.
Conclusions: A defined cycle of Cb-infection in dairy cattle farms consisting of silent, acute and chronic stage is proposed: Cb-shedding at parturition results in seronegative/IFN-γ-positive (immune) calves. 2-3 years later (!) these immune animals are introduced into the dairy cow herd and thereby increasing the level of herd immunity which is supposed to limit infection (shift to silent stage). Subsequently calves get not infected any longer and are raised as susceptible replacement heifers. Infection of susceptible cows again results in acute infection and PhII-antibodies in in these animals. Some animals in this group subsequently develop PhI-antibodies are prone to become chronically infected (PhI-antibodies, milk qPCR-positive). These chronically infected cows are regarded as a Cb-reservoir to maintain Cb in periods of increased herd-level immunity.

Presentation: Monday, 6 March 2017 from 8:30 - 8:45 in room 6.

043/ZOV

The Q fever pathogen *Coxiella burnetii* - to reveal the queries.

D. Frangoulidis*[1], M. Walter[1]
[1]*InstMikroBioBw, Munich, Germany*

Question: Although multiple efforts are done worldwide to elucidate the tricks of *C. burnetii* in pathogenesis and virulence it is still not really understood how this zoonotic pathogen could result in so different morphological, immunological and clinical features. The growing amount of genomic studies and datasets covers not only the genome but also more and more transcriptomic and metagenomic information. But still today one important comparison was missing – the genomic difference between the so called virulent Phase 1 strain Nine Mile RSA493 (clone 7), published first in 2003, and the avirulent Phase 2 strain Nine Mile RSA439 (clone 4). The genomic comparison between these two strain variants should be mandatory for the understanding of pathogenicity of *C. burnetii*

Methods: After cultivation in Buffalo Green Monkey cells, heat and chemical inactivation, DNA preparation was done with the Roche MagNA Pure compact system. First, a complete resequencing of the RSA493 with PacBio was performed. Then, a de novo sequencing of RSA439 with a combination of PacBio and Illumina HiSeq was done. The resulting data was assembled with HGAP3 and polished with Illumina reads using the pilon software. Afterwards, whole genome alignment with the public reference sequence and curation took place. Finally, a whole genome comparison of corrected RSA493 and RSA439 was carried out. Additionally, multiple sequence alignments of all regions/genes containing mutations were performed across all sequenced *C. burnetii* genomes to determine the conservation of the variations.

Results: Compared to the reference sequence, both newly sequenced genomes have 34 variations in common, whereof 7 are SNPs, 26 are small InDels and one insertion of 177 bp. Only five mutations are intragenic (one missense, four nonsense), whereas the other mutations are almost associated to IS1111 insertion elements or their possible insertion sites. Hence, these common variations do not contribute to the attenuation.

Conclusion: For the first time, we could now offer the completed whole genome sequence of this special variant of *C. burnetii*. We could confirm earlier findings about differences, especially a large 25 kb deletion, placed in genes involved in lipopolysaccharide synthesis. Knowing that phase variation is one explanation for pathogenicity in *Coxiella*, formerly this finding was postulated as the causal difference, but further studies could not prove this. Therefore, other co-factors were assumed. Our study identified 26 clone 4 specific mutations, including two candidate missense SNPs in two different proteins as well as one deletion of 245 bp within an uncharacterized membrane protein of 589 aa. Conservation analysis showed that this protein was also deleted in some other virulent strains (e.g. Q212, RSA331 and the Netherlands outbreak strains). Probably, it was just deleted in the context of reductive evolution. So, this variation could not explain the pathogenic difference. Focusing our further analysis and studies now on the remaining mutations will be the next – maybe final – step to reveal the pathogenic secrets of *C. burnetii.*

Presentation: Monday, 6 March 2017 from 8:45 - 9:00 in room 6.

044/ZOV

VIM-1 carbapenemase producing *Escherichia coli* isolated from retail seafood in Germany

N. Roschanski*[1], S. Guenther[1], T. T. T. Vu[2], J. Fischer[3], T. Semmler[4], S. Huehn[2,5], T. Alter[2], U. Roesler[1]
[1]*FU-Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany*
[2]*FU-Berlin, Institute of Food Safety and Hygiene, Berlin, Germany*
[3]*Federal Institute for Risk Assessment, Department Biological Safety, Berlin, Germany*
[4]*Robert Koch Institute, Berlin, Germany*
[5]*Beuth Hochschule, Life Science and Technology, Berlin, Germany*

Introduction/ Objectives: Carbapenems belong to the group of last choice antibiotics in human medicine. Therefore, the increasing number of reports describing carbapenemase-producing Enterobacteriaceae is worrying. Within the last couple of years it was shown that the occurrence of carbapenemase-producing bacteria is no longer limited to clinical settings and carbapenemase-producing bacteria have been isolated from environmental surroundings as well as wild birds, companion- and food-producing animals all over the world. This trend depicts an important topic for the public health sector. In 2013 the EU legislation implemented the monitoring of carbapenemase-producing *Salmonella* spp. and *E. coli* in food-producing animals (chicken, turkey, pigs and cattle) and meat samples (2013/652/EU). However, none of the frequently raw consumed food products like vegetables, fruits or seafood have been included. In the here described study 160 seafood samples derived from 12 retail markets in Berlin, Germany (sampled from December 2015 to August 2016) were investigated for the presence of carbapenemase-producing Enterobacteriaceae.

Material & methods: A set of 45 Enterobacteriaceae isolated from seafood samples (clams and shrimps) were investigated for the presence of the carbapenemase genes *bla*VIM, *bla*NDM, *bla*OXA-48, *bla*KPC and *bla*GES using real-time PCR. Positive isolates were further investigated by MIC determination and whole genome sequencing (using MiSeq Reagent v3 600-cycle Kit, 2 × 300 cycles – Illumina), followed by a subsequent data analysis using the CGE platform (http://www.genomicepidemiology.org/). In addition a more detailed analysis of the carbapenemase containing plasmid was performed (S1-PFGE and transformation experiments followed by WGS sequence analysis).

Results: Out of the 45 investigated isolates, one *E. coli* of ST10 derived from a Venus clam (*Tapes semidecussatus*) - harvested in the Mediterranean Sea - contained *bla*VIM-1. This gene was part of the variable region of a class I integron accompanied by the resistance genes *aacA4*, *aphA15*, *aadA1*, *catB2* as well as *sul1*. Beside the class 1 integron, the isolate possessed several additional resistance genes including the extended-spectrum beta-lactamase *bla*SHV-12, the fluoroquinolone resistance gene *qnrS1* (both of them co-located on the same IncY plasmid) as well as the AmpC gene *bla*ACC-1.

Conclusion: The presence of carbapenemase-producing Enterobacteriaceae in seafood from a German retail market is alarming and emphasizes the importance of obligatory monitoring programs within the food production chain as well as the development of concomitant intervention strategies, regulating the prudent use of antibiotics as well as the containment of an environmental spread of the resistant bacteria in both, animals and humans.

Presentation: Monday, 6 March 2017 from 9:00 - 9:15 in room 6.

045/ZOV

Occurrence of carbapenem-resistant *Klebsiella pneumoniae* in broiler poultry farming and humans in contact in Egypt

E. Hamza*[1], S. Dorgham[2], D. Hamza[1]
[1]*Faculty of Veterinary Medicine, Cairo University, Zoonoses, Giza, Egypt*
[2]*National Research Institute, Microbiology and Immunology, Cairo, Egypt*

Carbapenems are broad-spectrum beta lactam antibiotics that are considered as the last-line therapy against multidrug-resistant Gram-negative bacteria. Nowadays, the increasing number of multi-drug-resistant (MDR)-*Klebsiella pneumoniae* (KP) is a major challenge for human medicine with fatal outcome. Of these, carbapenem-resistant-KP (CRKP) are of particular concern. The most remarkable mechanism of resistance in CRKP is the production of carbapenemases as KPC, NDM, VIM and OXA-48-like. Carbapenemases-encoding-genes possess an exceptional potential for dissemination and their precise origin remains undefined. Therefore, our objectives were to investigate the occurrence of CRKP strains in broiler chickens, drinking water and humans working in contact with chickens and to identify the carbapenem resistance determinants among isolates from different sources. Organs and droppings were collected from 100 broilers with signs of respiratory disease at five broiler farms in Giza Governorate, Egypt. In addition, 20 drinking water samples and 49 faecal samples from workers and veterinarians working at these farms were included in the study. After culture on MacConkey agar, suspected *K. pneumoniae* colonies were identified by phenotypic tests. Susceptibility to carbapenems was tested in confirmed *K. pneumoniae* isolates by disc diffusion and the resistant isolates were subjected to PCR for detection of carbapenemase-encoding genes *blaKPC*, *bla*OXA-48 and *bla*NDM. Our results showed isolation of *K. pneumoniae* from 35% broilers and 25% water samples. Out of the 35 isolates from poultry, 15 were carbapenem-resistant with all of them were *bla*NDM positive including 11 isolates harbouring *blaKPC*, *bla*OXA-48 and *bla*NDM genes, and four containing either *blaKPC* and *bla*NDM (n=3) or *bla*OXA-48 and *bla*NDM (n=1). Similarly, three out of the five *K. pneumoniae* isolates from drinking water were positive for *blaKPC* and *bla*NDM (n=1) or the three genes (n=2). Interestingly, 55.5% of the *K. pneumoniae* isolates from humans displayed carbapenem-resistance and all of them were positive for the three genes. In conclusion, carbapenemase-producing *K. pneumoniae* occurred at relatively high frequency among broilers, drinking water and workers at poultry farms in Egypt. The high frequency of *bla*OXA-48 and *bla*NDM CRKP isolates in the public community confirms their endemic presence in Egypt.

Presentation: Monday, 6 March 2017 from 9:15 - 9:30 in room 6.

046/ZOV

Genetic Relatedness of CTX-M producing *E. coli* from human, animal and poultry in Pakistan

S. Raza*[1], A. Ali[2], M. Mohsin[1]
[1]*University of Agriculture Faisalabad, Microbiology, Faisalabad, Pakistan*
[2]*National Institute of Biotechnology and Genetic Engineering (NIBGE), Health Biotechnology, Faisalabad, Pakistan*

Background: Antimicrobial resistance (AMR) is an ever-growing problem for communities and multiple sectors. Antimicrobial resistance bacteria are causing infections in humans and animals, and are transmitted through food chain. Proposed study was aimed for genetic diversity between extended spectrum beta-lactamases (ESBL) producing *E. coli* isolates under One Health initiative.
Material and Methods: A total of 150 samples, including humans (n=50), cattle (n=50) and poultry (n=50), were collected. All samples were cultured on CHROM agar and Clinical Laboratory Standard Institute CLSI-2012 criteria was used for screening of ESBL producing *E. coli* isolates followed by Vitek-2 system for determining the antimicrobial profile of ESBL isolates. Clinically important *blaCTX*-M was detected by PCR and sequencing of the CTX-M producing *E. coli* was done. Phylogrouping and replicon typing was done. BOX-PCR and PFGE were performed for detection of clonal relatedness.
Results: A total of 29 CTX-M containing ESBLs *E. coli* were isolated from human clinical isolates (32%), cattle (18%) and poultry (8%). Maximum resistance was found against tetracycline, doxycycline and sulfamethoxazole. 27/29 isolates were resistant to more than 3 class of antibiotics and were termed as multidrug resistant (MDR). Sequencing of the CTX-M producing *E. coli* isolates revealed CTX-M-15(25/29) was the most common type. F-lA and F-ll genes were most prevalent plasmid types while phylogenetic group A and B1 were common. Two distinct clonal groups were found after PFGE and BOX-PCR analysis.
Conclusion: To best of our knowledge it is the first report on genetic diversity of ESBL producing *E. coli* from huamans animals and poultry. The results of the study suggest that the presence of CTX-M-15 producing *E. coli* in each component of One Health is worrisome for health practitioner and policy makers.

Presentation: Monday, 6 March 2017 from 9:30 - 9:45 in room 6.

047/ZOV

Extended-Spectrum beta-lactamase-producing *Escherichia coli* isolated from horses at hospital admission: a challenge for veterinary infection control

B. Walther*[1], K. S. Klein[2], T. Semmler[3], A. K. Barton[2], S. Guenther[4], A. Luebke-Becker[1], H. Gehlen[2]
[1]*Freie Universität Berlin, Institut of Microbiology and Epizootics, Berlin, Germany*
[2]*Freie Universität Berlin, Equine Clinic, Surgery and Radiology, Berlin, Germany*
[3]*Robert Koch Institut, Berlin, Germany*
[4]*Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany*

Introduction: Zoonotic multi-drug resistant (MDR) pathogens such as extended-spectrum-beta-lactamase producing *Escherichia coli* (ESBL-Ec) were frequently associated with severe infections in hospitalized horses. Moreover, colonized equine patients entering the clinic might serve as a source of infection for themselves and others, since the stable environment provides generally more possibilities for bacterial spread compared with other clinical settings. Consequently, there are raising questions concerning biosafety and infection control apparent in equine clinics.
Material and Methods: A prospective study lasting six months was conducted at the clinic for horses at the Freie Universität Berlin in 2014 and 2015, respectively. Equine patients showing clinical signs and symptoms associated with either colic or open injury at hospital admission were included. Inclusion criteria for patient samples were stated as follows: nasal swabs of both anterior nostrils taken on arrival without any delay and fecal samples of first defecation after admission within 120 min. Fecal samples and nostril swabs were initially cultured on chromogenic selective screening plates for ESBL-producing Enterobacteriaceae. Suspect colonies were confirmed as ESBL-Ec by use of VITEK 2 GN ID card (bioMérieux) and subsequent ESBL confirmatory test was performed and interpreted according to Clinical & Laboratory Standards Institute (CLSI) standards. Molecular typing of ESBL-Ec included genomic macrorestriction using XbaI followed by pulsed-field gel electrophoresis (PFGE) pattern analysis, multi-locus sequence typing (MLST) and PCR for detection of *bla*SHV, *bla*TEM and *bla*CTX-M encoding sequences.
Results: In total 341 equine patients were enrolled in this study. 318 valid fecal samples were obtained, revealing an isolation rate

of 10.1% ESBL-Ec (32), while three (0.7%) of the nasal swabs (n= 340) were positive for ESBL-Ec.
Since some samples were positive for more than one ESBL-Ec phenotype, the isolate number does not match with the number of positive specimens. Comparative PFGE analysis showed a broad heterogeneity within the collection, mirrored by the detected STs (e.g. ST224, ST1683, ST539, ST1730, ST826 and novel STs). Interestingly, horses were found positive for a particular ESBL-Ec at different body sites. ESBL-EC isolates from different horses sharing a single PFGE pattern belonging to ST224 were also detected, even if administered in distinct years. Among the isolate collection, the *bla*CTX-M group was dominating.
Conclusion: Here, we provide evidence for a massive introduction of ESBL-Ec into a large clinical setting for equine patients in Germany, clearly depicting the current challenges for veterinary infection control and work place safety.

Presentation: Monday, 6 March 2017 from 9:45 - 10:00 in room 6.

048/INV
The human microbiome and its potential for understanding health and disease
K. E. Nelson*[1]
[1]*J. Craig Venter Institute (JCVI), Rockville, Maryland, United States*

No abstract has been submitted.

Presentation: Monday, 6 March 2017 from 11:00 - 11:30 in the Franconia Hall.

PLENARY 01
Microbiome and Commensals
06 March 2017 • 11:00 – 12:30

049/INV
Microbiota-mediated defense against intestinal infection
E. G. Pamer*[1]
[1] *Infectious Diseases Service, Memorial Sloan Kettering Cancer Center New York, New York, United States*

Infections caused by antibiotic-resistant bacteria generally begin with colonization of mucosal surfaces, in particular the intestinal epithelium. The intestinal microbiota provides resistance to infection with highly antibiotic-resistant bacteria, including Vancomycin Resistant *Enterococcus* (VRE) and *Clostridium difficile*, the major cause of hospitalization-associated diarrhea. Metagenomic sequencing of the murine and human microbiota following treatment with different antibiotics is beginning to identify bacterial taxa that are associated with resistance to VRE and *C. difficile* infection. We demonstrate that reintroduction of a diverse intestinal microbiota to densely VRE colonized mice eliminates VRE from the intestinal tract. While oxygen-tolerant members of the microbiota are ineffective at eliminating VRE, administration of obligate anaerobic commensal bacteria to mice results in a billion-fold reduction in the density of intestinal VRE colonization. Many antibiotics destroy intestinal microbial communities and disable the native microbiota's ability to inhibit *C. difficile* growth and toxin production. Which intestinal bacteria provide resistance to *C. difficile* infection and their *in vivo* inhibitory mechanisms remains unclear. By treating mice with different antibiotics that result in distinct microbiota changes and lead to varied susceptibility to *C. difficile*, we correlated loss of specific bacterial taxa with development of infection. Mathematical modeling augmented by microbiota analyses of hospitalized patients identified resistance-associated bacteria common to mice and humans. Using these platforms, we determined that *Clostridium scindens*, a bile acid 7-dehydroxylating intestinal bacterium, is associated with resistance to *C. difficile* infection and, upon administration, enhances resistance in a secondary bile acid-dependent fashion. Using a workflow involving mouse models, clinical studies, metagenomic analyses and mathematical modeling, we identified a probiotic candidate that corrects a clinically relevant microbiome deficiency. Our studies indicate that obligate anaerobic bacteria enable clearance of intestinal VRE colonization and may provide novel approaches to prevent the spread of highly antibiotic-resistant bacteria.

References
[1] Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A, Littmann E, van den Brink MR, Jenq RR, Taur Y, Sander C, Cross J, Toussaint NC, Xavier JB, Pamer EG. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature. (2015) 517(7533):205-208.
[2] Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, No D, Gobourne A, Viale A, Dahi PB, Ponce DM, Barker JN, Giralt S, van den Brink M, Pamer EG. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood. (2014) 124(7):1174-82.
[3] Taur Y, Xavier JB, Lipuma L, Ubeda C, Goldberg J, Gobourne A, Lee YJ, Dubin KA, Socci ND, Viale A, Perales MA, Jenq RR, van den Brink MR, Pamer EG. Intestinal domination and the risk of bacteremia in patients undergoing allogeneic hematopoietic stem cell transplantation. Clin Infect Dis. 2012 Oct;55(7):905-14.
[4] Ubeda C, Bucci V, Caballero S, Djukovic A, Toussaint NC, Equinda M, Lipuma L, Ling L, Gobourne A, No D, Taur Y, Jenq RR, van den Brink MR, Xavier JB, Pamer EG. Intestinal microbiota containing Barnesiella species cures vancomycin-resistant Enterococcus faecium colonization. Infect Immun. 2013 Jan 14.
[5] Caballero S, Pamer EG. Microbiota-mediated inflammation and antimicrobial defense in the intestine. Annual Review of Immunology. 2015; 33:227-258.

Presentation: Monday, 6 March 2017 from 11:30 - 12:00 in the Franconia Hall.

050/INV
Cancer as a disease of the metaorganism
G. Trinchieri*[1]
[1]*Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, United States*

Commensal microorganisms colonize barrier surfaces of all multicellular organisms, including those of humans. For more than 500 million years commensal microorganisms and their hosts have coevolved and adapted to each other. As a result, the commensal microbiota affects many immune and non-immune functions of their hosts, and de facto the two together comprise one metaorganism. The commensal microbiota communicates with the host via biologically active molecules. Recently, it has been reported that microbial imbalance may play a critical role in the development of multiple diseases, such as cancer, autoimmune conditions and increased susceptibility to infection. The commensal microbiota not only may affect the development, progression and immune evasion of cancer but it has also important effects on the response to cancer immune- and chemo-therapy. Myeloid cells are a major component of the tumor microenvironment where they play a dual role inducing anti-tumor immune responses but mostly promoting immune evasion, tumor progression and metastases formation. Myeloid cells respond to environmental factors including signals derived from

commensal microbes that modulate their function and reactivity thus impacting the response to cancer therapy.

Presentation: Monday, 6 March 2017 from 12:00 - 12:30 in the Franconia Hall.

HYGIENESYMPOSIUM 01
General and Hospital Hygiene
06 March 2017 • 12:45 – 14:00

No abstracts have been submitted.

PLENARY 02
Non-coding RNA
06 March 2017 • 14:30 – 16:00

054/INV
The temperature-responsive RNA structurome of a bacterial pathogen
F. Naberhaus*[1]
[1]*Microbial Biology, Ruhr University Bochum, Germany*

Structured mRNA segments affect gene expression in many ways. The formation of complex structures in the 5'-untranslated region (5'-UTR) of numerous heat shock and virulence genes controls translation in response to temperature (1). Such RNA thermometers trap the ribosome binding site (RBS) by formation of a secondary structure. An increase in temperature to 37°C (virulence genes) or higher (heat shock genes) destabilizes the structure in a reversible, zipper-like manner. Liberation of the RBS permits formation of the translation initiation complex.
The human pathogens *Vibrio cholerae* and *Yersinia pseudotuberculosis* use RNA thermometers to control synthesis of their master virulence regulators ToxT and LcrF, respectively (2,3). Strains with a stabilized "closed" RNA structure are unable to infect mice demonstrating that a functional thermosensor is essential for pathogenicity.
Next-generation sequencing techniques have the potential to probe RNA structures on a global scale (4, 5). By Parallel Analysis of RNA Structures (PARS), we mapped the RNA structurome of *Y. pseudotuberculosis* at three different temperatures (25°C = free-living, 37°C = in the host; 42°C = heat shock) and found that average mRNAs tend to be unstructured around the ribosome binding site (6). A number of 5'-UTRs and intercistronic regions deviating from this general principle were shown to contain temperature-responsive RNA elements. This study demonstrates the power of high-throughput RNA structure probing approaches to identify new sensory and regulatory RNA structures.

References
[1] Kortmann J, Narberhaus F. 2012. Bacterial RNA thermometers: Molecular zippers and switches. *Nature Rev Microbiol* 10:255-265.
[2] Weber GG, Kortmann J, Narberhaus F, Klose KE. 2014. An RNA thermometer controls temperature-dependent virulence factor expression in *Vibrio cholerae*. *PNAS* 111:14241-14246.
[3] Böhme K, Steinmann R, Kortmann J, Seekircher S, Heroven AK, Berger E, Pisano F, Thiermann T, Wolf-Watz H, Narberhaus F, Dersch P. 2012. Concerted actions of a thermo-labile regulator and a unique intergenic RNA thermosensor control *Yersinia* virulence. *PLoS Pathog* 8:e1002518.
[4] Wan Y, Kertesz M, Spitale RC, Segal E, Chang HY. 2011. Understanding the transcriptome through RNA structure. *Nature Rev Genet* 12:641-655.
[5] Wan Y, Qu K, Ouyang Z, Kertesz M, Li J, Tibshirani R, Makino DL, Nutter RC, Segal E, Chang HY. 2012. Genome-wide measurement of RNA folding energies. *Mol Cell* 48:169-181.
[6] Righetti F, Nuss AM, Twittenhoff C, Beele S, Urban K, Will S, Bernhart SH, Stadler PF, Dersch P, Narberhaus F. 2016. The temperature-responsive *in vitro* RNA structurome of *Yersinia pseudotuberculosis*. *PNAS* 113:7237-7242.

Presentation: Monday, 6 March 2017 from 14:30 - 15:00 room Franconia.

055/INV
J. Vogel*[1]
[1]*Julius Maximilians University of Wuerzburg, Institute for Molecular Infection Biology, Wuerzburg, Germany*

No abstract has been submitted.

Presentation: Monday, 6 March 2017 from 15:00 - 15:30 room Franconia.

056/INV
Regulation of antibiotic resistance via non-coding RNAs
R. Sorek*[1]
[1]*Weizmann Institute of Science, Department of Molecular Genetics, Rehovot, Israel*

Riboswitches and attenuators are cis-regulatory RNA elements, most of which control bacterial gene expression via metabolite-mediated, premature transcription termination. We developed an unbiased experimental approach for genome-wide discovery of such ribo-regulators in bacteria. Using this approach, we detected numerous antibiotic responsive ribo-regulators that control antibiotic resistance genes in pathogens and in the human microbiome.

Presentation: Monday, 6 March 2017 from 15:30 - 16:00 room Franconia.

PLENARY 03
Refugee and Migrant Health
06 March 2017 • 14:30 – 16:00

057/INV
Does migration affect infectious disease epidemiology in Germany?
A. Gilsdorf*[1]
[1]*Robert-Koch-Institute, Berlin, Germany*

In 2015/2016, the sudden influx of migrants raised multiple concerns regarding its impact on the epidemiology of infectious diseases in Germany. However, very little data was available to respond to these concerns. Up to 2015, the mandatory notification system for infectious diseases in Germany did not generally collect the information if a case of a notifiable disease was an asylum seeker or not, as data minimization is one of its principles. To assess the epidemiological situation of infectious diseases among asylum seekers, in week 40/2015 we started collection of additional information on being an asylum seeker, country of birth, date of entry into Germany and living in a group accommodation for cases with infections that require notification by name.
The Robert Koch Institute (RKI) publishes a monthly report on this data. In the first 48 weeks of 2016, 6.096 cases of infectious diseases among asylum seekers have been reported to RKI, representing 2% of the overall notifications in Germany (as of 20.12.2016). The number of cases who were known to be asylum seekers has dropped from around 250 cases/per week in January to 60-80 cases per week in November 2016.
We see predominantly cases of vaccine-preventable and gastro-intestinal diseases which are generally preventable by early vaccination and better hygiene. In Germany a routinely screening

amongst asylum seekers is in place for some diseases including tuberculosis, hepatitis B and hepatitis C. As expected, these diseases are reported more frequently. Otherwise for Germany unusual diseases (e.g. louse-borne relapsing fever) are seen only sporadically. Regular notification data also show an increase of cases of HIV and Malaria over the last years. Both diseases are notified anonymously directly to RKI.

Presentation: Monday, 6 March 2017 from 14:30 - 15:00 in the Barbarossa Hall.

058/INV
Challenges in the management of infectious diseases in migrants and refugees
A. Stich*[1]
[1]*Missionsärtzliche Klinik Wuerzburg, Wuerzburg, Germany*

The influx of refugees to Europe in the last years and the already existing high percentage of migrants in Germany result in an increasing importance of diseases which are poorly known. Systematic screening and routine medical care reveal a relatively high prevalence of infectious diseases in migrant populations. Their diagnosis and management require special expertise, sometimes even special protections or isolation procedures. However, refugees and migrants are not a public health threat to the general population. Instead they are a very vulnerable group and require increased and specialized attention.

Presentation: Monday, 6 March 2017 from 15:00 - 15:30 in the Barbarossa Hall.

059/INV
Infectious Disease aspects of migrant health
A. Ammon*[1]
[1]*European Centre for Disease Prevention and Control, Stockholm, Sweden*

No abstract has been submitted.

Presentation: Monday, 6 March 2017 from 15:30 - 16:00 in the Barbarossa Hall.

MINISYMPOSIA 09
Concepts to tackle complexity: applying theory to microbial communities (FG UM)
06 March 2017 • 18:00 – 19:45

060/UMV
Ecological keystones drive disease dynamics in the lung microbiome of persons with cystic fibrosis
S. Widder*[1]
[1]*Medical University of Vienna, Department of Medicine 1, Vienna, Austria*

Microbes are everywhere and make up most of the biomass on earth. Frequently, they form microbial communities (MCs) and conduct complex, collective functions that are of highest importance for biogeochemical cycles on earth and human well-being alike. For example, the human gut microbiome can actively promote human health or be etiologic for chronic diseases or cancer. These emergent community functions are driven by microbial interactions. To build predictive understanding and manage microbial functions for the human context, research needs to address all scales involved from metabolic interactions up to ecological roles and community dynamics. In my talk I will present our modeling approach that allows detection of keystone species from NGS data. Such keystones are not only relevant for community persistence, but are also prime targets for improving human health. I will show how networks and graph theory are applicable for pinpointing the dynamics of the human microbiome in airways of cystic fibrosis (CF) patients and how our generic framework enables prediction of drug targets in metabolic networks of the CF microbiome. Moreover, the presented concepts are directly transferable to other lung disorders with poly-microbial implication, such as COPD or asthma.

Presentation: Monday, 6 March 2017 from 18:00 - 18:30 in the Franconia Hall.

061/UMV
Application of Rank Abundance Distributions (RADs) in study of next generation sequencing data: Uncovering the hidden information in abundance data
M. Saeedghalati*[1], F. Farahpour[1], D. Hoffmann[1]
[1]*University of Duisburg-Essen, Biology, Essen, Germany*

Rank Abundance Distributions (RADs) have been widely used in ecology to study the properties of ecological communities but they have been scarcely employed in the high-throughput sequencing experiments. RADS as the sorted abundance vector of communities contain all information in abundance data and are potential to be used as a community descriptor, located in the intermediate level of complexity [1,2], i.e. comprising the whole vector of abundances, they are more complex than univariate ecological measures such as richness, evenness, etc. but dropping the species label makes them simpler than the methods working with both abundance and taxonomy data.
The common approach for dealing with RADs is model fitting which has been successfully used in some ecological studies but in general, RADs from different communities can not be adequately described by simple mathematical models with few parameters, e.g. a single log-normal distribution. However in macroecology, knowledge about the properties and relations between the observed animals and plants can be used to deconstruct multi-modal distributions, and to fit simple models to fractions of the samples [1,3]. This knowledge is generally not available for high-throughput sequencing data of complex genetic communities, so that RAD analyses based on simple parametric models are difficult. This calls for a non-parametric approach in study of RADs.
Here we introduce a new normalization method (MaxRank normalization) for study of RADs which can turn them into a generic, expressive descriptor for quantitative comparison of communities in many areas of biology. The approach is essentially non-parametric and allows for the direct quantitative comparison of complex RADs without deconstruction and model fitting. By applying this method RADs can be used as an analytic tool to generate easily interpretable results, and also as a basis for quantitative models. To illustrate the versatility of the method, we have analyzed RADs from various genetic communities, i.e. assemblages of genetically diverse cells or organisms, including human B cells, gut microbiomes under antibiotic treatment and of different ages and countries of origin, and other human and environmental microbial communities.
We show that normalized RADs enable us to use quantitative approaches, like clustering, ordination or classification, that help to understand structures and dynamics of complex genetic communities.

References
[1] McGill, Brian J., et al. "Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework." Ecology letters 10.10 (2007): 995-1015.
[2] Foster, Scott D., and Piers K. Dunstan. "The analysis of biodiversity using rank abundance distributions." Biometrics 66.1 (2010): 186-195.
[3] Magurran AE. Measuring Biological Diversity. Wiley; 2004.

Presentation: Monday, 6 March 2017 from 18:30 - 18:45 in the Franconia Hall

062/UMV

From monitoring to microbiome management with microbial cytomics

C. Koch*[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany*

The fast dynamics of natural communities regarding activity and composition are a challenge for microbial community monitoring and even more for the prediction and understanding of their functions. Microbial cytomics has the potential to access microbial community behaviour at a time resolution smaller than ever before. At the same time, the underlying ecological principles regarding diversity and richness, community structure and functional dynamics can be investigated. Microbial flow cytometry is a convenient method for following fast dynamics in complex microbial communities based on high throughput single cell measurements. Within a few minutes 200,000 cells can individually be measured regarding intrinsic (cell size, morphology, and granularity) and extrinsic (marker molecules, e.g. DAPI for DNA staining) optical cell properties. Standardized protocols for different sample types of environmental and biotechnological habitats are available and objective and reproducible analyzing methods enable fast analysis, visualization and correlation with environmental parameters of the obtained data sets. The combination of microbial flow cytometry with other high throughput omics technologies bears an enormous potential for understanding microbial ecosystem functions and their potential prediction based on advancement in ecological theory.

References

[1] Koch, C., Harms, H., Müller, S., Dynamics in the microbial cytome - single cell analytics in natural systems, 2014, Curr. Opin. Biotechnol. 27, 134-141.
[2] Koch, C., Müller, S., Harms, H., Harnisch, F., Microbiomes in bioenergy production: From analysis to management, 2014, Curr. Opin. Biotechnol. 27, 65-72.

Presentation: Monday, 6 March 2017 from 18:45 - 19:00 in the Franconia Hall.

063/UMV

Dispersal modulates local environmental sorting in freshwater microbial metacommunities

M. M. Bengtsson*[1,2], T. Lumpi[2], L. Eggers[2], Z. Horvath[2], C. Preiler[2], R. Ptacnik[2]
[1]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*
[2]*WasserCluster Lunz, Lunz am See, Austria*

The pelagic zone of lakes harbors diverse communities of prokaryotic and eukaryotic microorganisms that participate in intricate networks of interactions, ranging from trophic and parasitic interactions to competition and facilitation. The assembly of these communities can be understood via metacommunity theory, as lakes are distinct habitat patches in the regional landscape connected by dispersal and each lake features local environmental factors that may select for adapted taxa. However, microorganisms have traditionally been considered not limited by dispersal, and their communities therefore mainly shaped by the local environment. We set out to examine the influence of dispersal and local environmental selection on experimental lake microbial communities and to identify potential biotic interactions between bacterial and microbial eukaryote taxa. Using mesocosms (320 L, n=40), we manipulated dispersal as well as nutrient regime and zooplankton presence (local environment) in a full factorial design and sampled on three occasions spanning a 4-week period. Illumina MiSeq PCR amplicon sequencing of the 16S and 18S rRNA genes revealed differences in microbial community composition and diversity depending on both dispersal and the local environment. Dispersal increased the diversity (taxon richness) of both bacterial and eukaryote communities. Zooplankton presence had the strongest direct effects on community composition, while the effect of dispersal rivaled that of nutrient enrichment on microbial communities. However, significant interactive effects of dispersal and both zooplankton presence and nutrient regime suggested that adaptation of communities to the local environment depends on immigration of new taxa. Co-occurrence network analysis highlighted biotic interactions that may underlie the ability of communities to adapt locally, such as interactions between putative parasitic fungi and phytoplankton. Our results clearly demonstrate that dispersal is a powerful and underestimated driver of freshwater microbial community dynamics that modulates the way communities adapt to the local environment.

Presentation: Monday, 6 March 2017 from 19:00 - 19:15 in the Franconia Hall.

064/UMV

Competition and facilitation between the marine nitrogen-fixing cyanobacterium *Cyanothece* and its associated bacterial community

V. S. Brauer*[1,2,3], M. Stomp[1], T. Bouvier[3], E. Fouilland[3], C. Leboulanger[3], V. Confurius-Guns[4], F. J. Weissing[2], L. J. Stal[1,4], J. Huisman[1]
[1]*University of Amsterdam, Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, Amsterdam, Netherlands*
[2]*University of Groningen, Department of Theoretical Biology, Center for Ecological and Evolutionary Studies, Groningen, Netherlands*
[3]*Université Montpellier 2, Laboratoire Ecologie des Systèmes Marins Côtiers ECOSYM, UMR 5119, CNRS, IRD, Ifremer, Montpellier, France*
[4]*Royal Netherlands Institute for Sea Research (NIOZ), Department of Marine Microbiology, Yerseke, Netherlands*

N2-fixing cyanobacteria represent a major source of new nitrogen and carbon for marine microbial communities, but little is known about their ecological interactions with associated microbiota. In this study we investigated the interactions between the unicellular N2-fixing cyanobacterium *Cyanothece* sp. Miami BG043511 and its associated free-living chemotrophic bacteria at different concentrations of nitrate and dissolved organic carbon (DOC) and different temperatures. High temperature strongly stimulated the growth of *Cyanothece*, but had less effect on the growth and community composition of the chemotrophic bacteria. Conversely, nitrate and carbon addition did not significantly increase the abundance of *Cyanothece*, but strongly affected the abundance and species composition of the associated chemotrophic bacteria. In nitrate-free medium the associated bacterial community was co-dominated by the putative diazotroph *Mesorhizobium* and the putative aerobic anoxygenic phototroph *Erythrobacter* and after addition of organic carbon also by the Flavobacterium *Muricauda*. Addition of nitrate shifted the composition toward co-dominance by *Erythrobacter* and the Gammaproteobacterium *Marinobacter*. Our results indicate that *Cyanothece* modified the species composition of its associated bacteria through a combination of competition and facilitation. Furthermore, within the bacterial community, niche differentiation appeared to play an important role, contributing to the coexistence of a variety of different functional groups. An important implication of these findings is that changes in nitrogen and carbon availability due to, e.g., eutrophication and climate change are likely to have a major impact on the species composition of the bacterial community associated with N2-fixing cyanobacteria.

Presentation: Monday, 6 March 2017 from 19:15 - 19:30 in the Franconia Hall.

065/UMV
Metabolic heterogeneity and symbiotic interaction of microbial communities in interconnected groundwater aquifers
A. J. Probst*[1], B. Ladd[1], J. Jarret[1], C. M. K. Sieber[1], J. Emerson[1], B. C. Thomas[1], M. Stieglmeier[1], A. Klingl[1], T. Woyke[1], M. C. Ryan[1], J. F. Banfield[1]
[1]UC Berkeley, Department of Earth and Planetary Sciences, Berkeley, United States

The subsurface hosts a massive reservoir of inorganic carbon, which supports complex microbial life. However, the diversity, functions and spatial heterogeneity of microbial communities in subsurface ecosystems are underexplored. Here, we investigated microbial communities in subsurface fluids from Crystal Geyser, Utah, a cold, CO2-driven geyser that sources groundwater from several different aquifer formations with varying depths throughout its 5-day eruption cycle. Using genome-resolved metagenomics and single cell genomics, we conducted an extensive genomic sampling of microorganisms spanning more than 1200 high-quality genomes belonging to 505 different bacterial and archaeal species. One highly abundant archaeon, a novel phylum-level lineage, was inferred to be the symbiotic partner of the most dominant carbon-fixing organism in the system. Continuously monitored water pressure and electrical conductivity, combined with geochemical analyses, were coupled to microbial source tracking throughout the 5-day eruption cycle of the geyser to reveal different microbial communities associated with sediment aquifers of different depths. Designated symbionts of the bacterial Candidate Phyla Radiation and archaeal members of the DPANN radiation were enriched in the deepest aquifer. Groundwater from different aquifers was also associated with specific metabolic capacities regarding C, N and S cycling, which included nitrogen fixation in the shallowest aquifer and sulfate reduction in the deepest aquifer. Interestingly, we identified three different carbon fixation pathways, which fluctuated in abundance with the cycling of the geyser. We further investigated the association of different carbon fixation pathways with different aquifers across six geographically distinct subsurface ecosystems using more than 2000 reconstructed genomes. The results imply that there are hotspots of CO2 fixation in the subsurface, which are frequently mediated by a single dominant species. The findings presented in this communication shed new light onto the genetic and metabolic versatility of microorganisms in the subsurface and reveal a so far unprecedented resolution of spatial and functional heterogeneity in these ecosystems.

Presentation: Monday, 6 March 2017 from 19:30 - 19:45 in the Franconia Hall.

MINISYMPOSIA 10
Interkingdom Signaling (FG RS)
06 March 2017 • 18:00 – 20:00

066/RSV
Show me the way: ethanolamine sensing is a bacterial GPS for navigating host niches
M. M. Kendall*[1]
[1]University of Virginia, School of Medicine, Charlottesville, United States

Chemical and nutrient signaling are fundamental for all cellular processes, including interactions between the mammalian host and the microbiota, which have a significant impact on health and disease. Ethanolamine is a component of phosphatidylethanolamine, a major phospholipid of mammalian and bacterial cell membranes, and thus is an abundant and essential molecule within the host. Our studies revealed a sophisticated mechanism in which bacteria exploit ethanolamine to gain specific information about the localized environment and modulate gene expression to overcome bacterial and host resistance mechanisms. For example, the food-borne pathogen *Salmonella* relies on ethanolamine metabolism in the intestine to sidestep nutritional competition from the resident microbiota and establish infection. Subsequently, *Salmonella* senses ethanolamine in the intracellular environment to deploy its type three secretion system-2, which enables *Salmonella* to replicate and disseminate throughout the body. The molecular mechanisms of ethanolamine-dependent gene regulation and niche adaptation, including our new findings, will be presented.

Presentation: Monday, 6 March 2017 from 18:00 - 18:30 in the Barbarossa Hall.

067/RSV
Studies on inter-species and inter-kingdom signaling in plant-associated bacteria
V. Venturi*[1]
[1]International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

Most bacteria living in the wild are part of complex multispecies communities indicating that interspecies communication is taking place. Moreover, bacterial communities living in close association with the plant are likely to undergo intensive inter-kingdom signaling. These aspects are now a major challenge in microbiology as microbial signaling has thus far been mainly studied in the laboratory in pure cultures. We are studying bacterial interspecies signaling using a plant disease as a model. The olive knot disease caused by *Pseudomonas savastanoi* results in tumors/galls in olive trees; we have established that inside the tumors, together with the pathogen, other bacterial species interact and communicate with the pathogen resulting in mutual benefit and in a more aggressive disease. We have also initiated studies to unravel interkingdom signaling mechanisms in *Pseudomonas syringae* and a potential novel class of signals has been discovered.

Presentation: Monday, 6 March 2017 from 18:30 - 19:00 in the Room 13.

068/RSV
Inter-kingdom-signaling via PAS4-LuxR receptors in *Photorhabdus luminescens*
J. Brehm[1], E. Rothmeier[1], S. Brameyer[1], R. Heermann*[1]
[1]Ludwig-Maximilians-Universität München, Biozentrum, Bereich Mikrobiologie, Martinsried/München, Germany

LuxR-like receptors are known to be involved in bacterial communication via acyl homoserine lactones (AHL). The prototypical AHL quorum sensing system consists of a LuxI-like AHL synthase and a cognate LuxR-like receptor, which senses the signal. Many proteobacteria possess further LuxR-family proteins that lack a cognate LuxI synthase. *Photorhabdus* species harbour a remarkably high number of those LuxR solos. However, the majority of the LuxR solos in all *Photorhabdus* species have a PAS4 instead of an AHL signal-binding domain. PAS4 domains share structural homology with PAS3 domains, which represent insect hormone binding domains in the fruit fly *Drosophila melanogaster*. As a first step to investigate the function of PAS-LuxR solos, we generated *P. luminescens* mutants lacking the entire gene clusters *plu0918-0925*, *plu2001-2016*, and *plu2018-2019*. Compared to the wild-type, these mutants exhibited different host specific decrease in pathogenicity, which has given first evidence that PAS4-LuxR receptors play a central role in sensing the specific insect host. Among others, *plu0258* was identified as a putative target gene of Plu2018 and/or Plu2019. Promoter activity of *plu0258* was inducible with *Galleria mellonella* insect homogenate in the wild-type, but not in the Δ*plu2018*/Δ*plu2019* mutant. This clearly showed that these PAS4-

LuxR receptors sense a signal, which is present in the insect host. Domain swapping of the PAS4 domain with the PAS3 domain of the *D. melanogaster* Met regulator resulted in PAS3-Plu2018 and PAS3-Plu2019 hybrid proteins, respectively, that responded to 20-hydroxyecdyson. Stability experiments with insect homogenate further revealed that the signaling molecule sensed by Plu2018/Plu2019 is a hormone-like substance.

Presentation: Monday, 6 March 2017 from 19:00 - 19:15 in the Room 13.

069/RSV

Intra-species and inter-kingdom signaling by the *Legionella pneumophila* α-hydroxyketone LAI-1

H. Hilbi*[1]
[1]*University of Zürich, Zürich, Switzerland*

Small molecule signaling promotes the communication between bacteria as well as between prokaryotes and eukaryotes. *Legionella pneumophila*, the causative agent of Legionnaires' disease, employs the α-hydroxyketone LAI-1 (3-hydroxypentadecane-4-one) for cell-cell communication (1). The opportunistic pathogen shows a bi-phasic life cycle, alternating between a replicative, non-virulent phase and a stationary, virulent phase. *L. pneumophila* employs the Lqs (*Legionella* quorum sensing) system as a major regulator of the growth phase switch.
The Lqs system produces, detects and responds to the signaling molecule LAI-1. The system comprises the autoinducer synthase LqsA, the cognate homologous sensor kinases LqsS and LqsT, as well as a prototypic response regulator, LqsR. LAI-1-regulated signaling through the sensor kinases converges on LqsR, which dimerizes upon phosphorylation (2, 3). The Lqs system regulates growth phase switch, pathogen-host cell interactions, bacterial motility, natural competence for DNA uptake, filament production and expression of a chromosomal "fitness island".
Interestingly, *lqsA* as well as synthetic LAI-1 also modulate the migration of eukaryotic cells in a dose-dependent manner (4). Inhibition of cell migration by LAI-1 occurs through a signaling pathway involving the scaffold protein IQGAP1, the small GTPase Cdc42 and a Cdc42 activator, the guanine nucleotide exchange factor ARHGEF9, but not other modulators of Cdc42, or other small GTPases. In summary, *L. pneumophila* LAI-1 not only regulates bacterial quorum sensing and the bi-phasic life cycle, but also mediates pathogen-host cell inter-kingdom signaling.

References

[1] Tiaden, A., Spirig, T. & Hilbi, H. Bacterial gene regulation by α-hydroxyketone signaling. *Trends Microbiol* 18, 288-297 (2010).
[2] Schell, U., Kessler, A. & Hilbi, H. Phosphorylation signaling through the *Legionella* quorum sensing histidine kinases LqsS and LqsT converges on the response regulator LqsR. *Mol Microbiol* 92, 1039-1055 (2014).
[3] Schell, U. *et al.* & Hilbi, H. The α-hydroxyketone LAI-1 regulates motility, Lqs-dependent phosphorylation signaling and gene expression of *Legionella pneumophila*. *Mol Microbiol* 99, 778-793 (2016).
[4] Simon, S. *et al.* & Hilbi, H. Inter-kingdom signaling by the *Legionella* quorum sensing molecule LAI-1 modulates cell migration through an IQGAP1-Cdc42-ARHGEF9-dependent pathway. *PLoS Pathog* 11, e1005307 (2015).

Presentation: Monday, 6 March 2017 from 19:15 - 19:30 in the Room 13.

070/RSV

Analysis of organic volatile compounds released by genetic variants of the two *Oxalobacteraceae* species *Duganella* and *Janthinobacterium* spp.

M. Himmel*[1,2], F. S. Haack[1], S. Rohn[3], W. R. Streit[1]
[1]*University of Hamburg, Microbiology and Biotechnology, Hamburg, Germany*
[2]*University of Hamburg, Center for Science and Peace Research, Hamburg, Germany*
[3]*University of Hamburg, Institute of Food Chemistry, Hamburg, Germany*

Introduction: The *Oxalobacteraceae* family consists of Gram-negative bacteria, which can colonize diverse environmental habitats like water, soil, or plants. Within this family, bacteria species of the genera *Janthinobacterium* and *Duganella* spp. are commonly found in soil and aquatic samples [1]. For the successful colonisation of such diverse habitats tightly coordinated cell-cell-communication via quorum sensing (QS) mechanisms is essential. In Gram-negative bacteria production and detection of acylated homoserine lactones is a predominant QS mechanism, whereas the *Janthinobacterium* QS system shows homologies to the CQS and LQS systems of *Vibrio cholerae* and *Legionella pneumophila* and relies on α-hydroxyketone-like autoinducer molecules as signal transmitters [2]. In addition, it has been experimentally shown for many bacterial species that they are able to release a variety of volatile organic compounds (VOCs), depending on the physiological status of the bacterial cell, some acting as signalling molecules [3]. Therefore, we used an adapted GC/MS method for the analysis of emitted VOCs in order to study the impact of genetic manipulations of the QS system in *Janthinobacterium* and *Duganella* bacterial strains.
Methods: Previous studies showed that the *Janthinobacterium* autoinducer molecule 1 (JAI-1) might play a critical role in the QS mechanism. To investigate the influence of JAI-1 on emitted VOCs, a deletion mutant was constructed, which lacks the JQS system autoinducer synthase JqsA required for JAI-1 production. This mutant and the corresponding deletion mutant of a closely related *Duganella* strain were used for the analysis of released VOCs by GC/MS. Bacteria were cultivated in liquid R2A medium under aerobic conditions, poured into sterile serum flasks and then sealed airtight. A headspace sample of accumulated volatile organic compounds was taken by using coated SPME fibres. Subsequently, desorption of VOCs and recording of GC peak retention times and mass fragmentation patterns were performed.
Results: Our initial screenings for characteristic volatile organic compounds released by *Janthinobacterium* and *Duganella* spp. revealed a number of VOCs, which could be used as markers for the biochemical characterisation of the bacterial cultures. Variations in GC peak intensities and the emergence of VOCs were observed which correspond to the genetic background of the strains analysed here.
Conclusion: The analysis of VOCs contributes to a better understanding of genetically induced manipulations of the QS system in bacteria of the *Oxalobacteraceae* family.

References

[1] Haack FS et al. Molecular keys to the *Janthinobacterium* and *Duganella* spp. interaction with the plant pathogen *Fusarium graminearum.* Front Microbiol, 26(7): article 1668, 2016.
[2] Hornung C et al. The *Janthinobacterium* sp. HH01 genome encodes a homologue of the *V. cholerae* CqsA and *L. pneumophila* LqsA autoinducer synthases. PLoS One, 8(2): e55045, 2013.
[3] Audrain B et al. Role of bacterial volatile compounds in bacterial biology. FEMS Microbiol Rev, 39(2): 222-33, 2015.

Presentation: Monday, 6 March 2017 from 19:30 - 19:45 in the Room 13.

071/RSV

The ApeRS Two-Component System of *Bacillus subtilis* Responds Exclusively to Antimicrobial Peptides of Eukaryotic Origin

K. Sievers[1], A. Adam[2], H. Gutzeit[2], D. Wolf*[1]
[1]*Technical University Dresden, General Microbiology, Dresden, Germany*
[2]*Technical University Dresden, Zoology, Dresden, Germany*

The cell envelope of bacteria represents the primary target of many antimicrobial substances, and especially of antimicrobial peptides (AMPs). Therefore, cell envelope integrity is crucial for survival of the cell. Bacteria evolved designated cell envelope stress response (CESR) mechanisms to constantly monitor and maintain the cell envelope integrity. The main part of the CESR is mediated by signal transduction via two-component systems (TCS) that link extracellular signal perception by a histidine kinase to a corresponding cellular response initiated by a response regulator [1]. In *Bacillus subtilis*, the CESR-mediating TCSs are mostly associated with ABC-transporters that confer resistance to antimicrobial compounds. The Bce-system that responds highly specific to bacitracin and several other structurally related AMPs has been extensively investigated [2]. Another Bce-like system of *B. subtilis* represents the ABC-transporter associated ApeRS TCS (formerly known as YxdJK). Here, the expression of the ABC-transporter ApeAB is controlled by the response regulator ApeR that binds to the P_{apeA} promoter. In the past, we tested a broad range of diverse AMPs (produced by prokaryotes) for induction of the Ape-system but we were unable to observe any response. Although it has already been described that the ApeRS TCS detects and responds to the human antimicrobial peptide LL-37 (cathelicidin) [3,4]. Recently, we have found a strong activation of the *apeA* promoter after treatment of *B. subtilis* with crude extract of the black soldier fly larvae *Hermetia illucens*. Microarray studies were performed and revealed a high expression of AMPs, e.g. cecropins and defensins in *H. illucens* larvae. Accordingly, we investigated the response of the Ape-system in the presence of *H. illucens* larvae extract and found that the Ape-system responds in a dose-dependent manner and confers resistance to the larvae extract. In addition to larvae extract purification, studies with additional synthetically produced defensins and cecropins of eukaryotic origin are currently in progress to find out more about the nature of the chemical compound that induces the Ape-system directly. Because of its inducer specificity, we hypothesize that the Ape-system exclusively responds to eukaryotic AMPs, which also led us to develop the Ape-system as a powerful biosensor for antibiotic research.

References

[1] Schrecke et al., 2012, In: Gross R, Beier D (eds) Two component systems in bacteria. Horizon Scientific Press, Hethersett, Norwich, UK, pp. 199-229
[2] Rietkötter et al., 2008, Mol Microbiol 68(3):768-85
[3] Joseph et al., 2004, Microbiology 150(8):2609-2617
[4] Pietiäinen et al., 2005, Microbiology 151(5):1577-1592

Presentation: Monday, 6 March 2017 from 19:45 - 20:00 in the Room 13.

MINISYMPOSIA 11
New enzymes and how to find them 1 (FG BT)
06 March 2017 • 18:00 – 19:30

072/BTV

Developing bacterial secondary metabolite producers via heterologous gene cluster expression

A. Loeschcke*[1], A. Domröse[1], R. Weihmann[1], K.-E. Jaeger[1,2], T. Drepper[1]
[1]*Heinrich-Heine-University Düsseldorf, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Forschungszentrum Jülich, Institute of Bio- and Geosciences Biotechnology (IBG-1), Jülich, Germany*

Questions: Bacterial secondary metabolites encompass diverse compounds with highly relevant bioactivities including antibiosis, cytotoxicity or immunosuppressive activity. To enable biotechnological access *via* heterologous biosynthesis in an amenable host, several critical steps have to be taken. Main challenges include the cloning, transfer, stable maintenance and the functional expression of all pathway genes. Moreover, the host cell metabolism is required to provide a suitable background for establishing a biosynthetic pathway, e.g. offering an appropriate precursor supply and tolerance towards the produced compound. Therefore, tools are required that enable the effective cloning of pathway genes and their expression in suitable bacterial hosts.
Methods: We have developed the molecular genetic tools TREX1 and yTREX that consist of gene cassettes enabling i) the straightforward cloning of large or multi-part gene clusters within *Saccharomyces cerevisiae via* homologous recombination, ii) the conjugational transfer of a TREX-labeled gene cluster into a broad range of bacterial hosts and iii) its stable integration into the host chromosome *via* transposition. iv) Finally, expression of the gene cluster can be realized by random integration downstream of a chromosomal promoter or by employment of convergent T7 RNA polymerase-mediated expression.
Results: By using different secondary metabolite pathway gene clusters for the production of carotenoids, prodiginines, violacein and phenazine that range in size from 6 to 21 kb, we could demonstrate the versatile applicability of TREX and yTREX: Straightforward one-step yeast recombination of multiple DNA fragments enabled rapid cloning of the natural and recombinant gene clusters. After stable genomic integration of a TREX-labeled gene cluster, we could show feasibility of convergent T7 RNA polymerase-based gene cluster expression in a range of bacterial hosts1. Moreover, using *Pseudomonas putida* as a suitable production chassis for antimicrobial compounds, random genomic integration enabled identification of naturally highly transcribed chromosomal regions suitable for high-level expression of heterologous pathway genes. This approach resulted in *P. putida* production strains that yield titers of prodigiosin2 and violacein in the 100 mg/L range.
Conclusion: The developed tools allow the reconstitution of gene cluster-encoded pathways within a number of different hosts in a plug-and-play fashion which offers new perspectives in the fields of genome mining and synthetic biology.

References

[1] Loeschcke A., Markert A., Wilhelm S., Wirtz A., Rosenau F., Jaeger K.-E., and Drepper T. (2013). TREX – A Universal Tool for the Transfer and Expression of Biosynthetic Pathways in Bacteria. ACS Synth. Biol. 2, 22–33
[2] Domröse, A., Klein, A., Hage-Hülsmann, J., Thies, S., Svensson, V., Classen, T., Pietruszka, J., Jaeger, K.-E., Drepper, T., Loeschcke, A. (2015). Efficient recombinant production of prodigiosin in *Pseudomonas putida*. Front. Microbiol. 6:972

Presentation: Monday, 6 March 2017 from 18:00 - 18:30 in room 10-11.

073/BTV
Engineering of CO2 fixing reaction cascades to synthesize a diverse library of polyketide extender units
B. Vögeli*[1], D. Peter[1], S. Benkstein[1], P. Gerlinger[1], T. J. Erb[1]
[1]*Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany*

Introduction: Crotonyl-CoA carboxylase/reductases (Ccrs) are a recently discovered class of enzymes. Ccrs catalyze the reductive carboxylation of a-b unsaturated enoyl-CoA esters into their corresponding (*S*)-alkylmalonyl-CoA derivatives1-2. In primary metabolism Ccrs serve as key enzymes in acetate assimilation pathways. In secondary metabolism Ccrs provide the building blocks for the biosynthesis of polyketides with a wide range of bioactivities. Here we extended the substrate scope of Ccrs for the production of novel, unnatural alkylmalonyl-CoA derivatives. We used these engineered enzymes in a proofreading enzyme reaction cascade to synthesize alkylmalonlyl-CoA ester with yields >90% on an mg scale. Finally, we demonstrated incorporation of these compounds into model polyketides *in vitro*.
Results: We screened more than 80 diverse Ccr´s and identified three residues that determine the substrate specificity in this enzyme family3. Based on this understanding, we changed the active site pocket of a fast, but non-promiscuous enzyme into a variant that produces a multitude of different alkylmalonyl-CoA compounds at catalytic efficiencies >105 M-1s-1. Large scale synthesis of alkylmalonyl-CoA derivatives with Ccrs poses multiple problems: **i)** Availability of unsaturated precursor acids for the chemical synthesis of the corresponding enoyl-CoA substrates is very limited. **ii)** The chemical synthesis of enoyl-CoA esters requires a purification step before enzymatic carboxylation. **iii)** all Ccrs possess an inherent side reaction that reduces the enoyl-CoA substrate, instead of carboxylating it (sometimes up to 90% even for its natural substrate). To overcome these problems we developed a synthetic route to alkylmalonyl-CoA ester starting from the more available and cheap saturated precursor acids, which can be chemically coupled to CoA under mild conditions. This saves the purification step. The saturated CoA-esters are directly desaturated and carboxylated in a one-pot reaction cascade containing a newly described promiscuous CoA-oxidase as well as a promiscuous Ccr. The CoA-oxidase desaturates the saturated CoA-ester to produce the enoyl-CoA substrate *in situ*. Additionally the CoA-oxidase serves as a proofreading enzyme that re-oxidizes the side-product of the Ccr, increasing alkylmalonyl-CoA yield dramatically.
Conclusion: We engineered promiscuous variants of crotonyl-CoA carboxylase/reductase and successfully used them in combination with proofreading CoA-oxidases to synthesize a variety of alkylmalonyl-CoA derivatives on an mg scale. We used these unnatural extender units to test and engineer the substrate specificity of AT domains in an *in vitro* polyketide synthase model system.

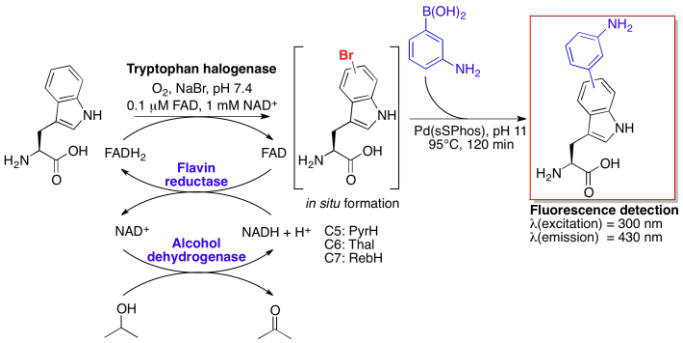
References
[1] Erb TJ, et al. PNAS 106, 8871-8876 (2009)
[2] Rosenthal RG, et al. Nature Chemical Biology, 10, 50-55 (2014)
[3] Peter DM, et al. Angewandte Chemie, 54, 13457–13461 (2015)

Presentation: Monday, 6 March 2017 from 18:30 - 19:00 in room 10-11.

074/BTV
Synthetic Application and Engineering of Flavin-Dependent Halogenases
C. Schnepel*[1], H. Minges[1], M. Frese[1], N. Sewald[1]
[1]*Organic and Bioorganic Chemistry, Department of Chemistry, Bielefeld University, Bielefeld, Germany*

Site-selective enzymatic halogenation proceeds under benign conditions in aqueous medium in presence of oxygen and halide salts, whereas conventional approaches usually require hazardous chemicals and show poor selectivity. Notably, haloarenes allow useful transformations like metal-catalyzed cross-couplings or nucleophilic substitutions.
Flavin-dependent tryptophan halogenases offer a broad synthetic utility owing to their intriguing regioselectivity, but the *in vitro* application is hampered by severe limitations: mainly low activity and narrow substrate profile constitute the major bottlenecks.[1,2] Initially, the tryptophan 7-halogenase RebH from *Lechevalieria aerocolonigenes* was employed for the regioselective halogenation of tryptophan and substituted derivatives.[3] By immobilization as cross-linked enzyme aggregates we were able to increase the stability of RebH resulting in scalable bromination of tryptophan.[4] Owing to the high selectivity later a one-pot cascade comprising *in situ* bromination and Suzuki-Miyaura cross-coupling was developed to form a set of 18 different C5-, C6-, and C7-aryl substituted N^{α}-Boc protected tryptophans.[5]
To overcome the inherent drawbacks of biohalogenation, we further emphasized on enzyme engineering.[6] Due to the lack of a facile high-throughput halogenase assay, Suzuki-Miyaura coupling was developed as the key step for mutant screening. Cross-coupling between a bromotryptophan and 3-aminophenylboronic acid gives a fluorescent biaryl that can be detected with high specificity in a microtiter plate. The reaction conditions were optimized for an application in *E. coli* crude lysate, in order to pave the way towards enzyme engineering. A mutant library of the tryptophan 6-halogenase Thal was generated by error-prone PCR and screened for improved thermostability. High-throughput screening with a fluorescence readout led to the identification of a Thal variant showing an increased denaturation temperature and higher activity. Currently, our efforts continue on optimizing halogenases towards chemoenzymatic synthesis. We desire to find novel variants with higher activity and an extended substrate scope to facilitate the integration of halogenases in multistep synthesis giving rise to sustainable synthetic routes.

Figure 1. Halogenase activity screening based on fluorogenic cross-coupling.



References
[1] C. Dong, S. Flecks, S. Unversucht, C. Haupt, K.-H. van Pée, J. H. Naismith, *Science* 2005, *309*, 2216–2219.
[2] V. Weichold, D. Milbredt, K.-H. van Pée, *Angew. Chem. Int. Ed.* 2016, *55*, 6374–6389.
[3] M. Frese, P. H. Guzowska, H. Voß, N. Sewald, *ChemCatChem* 2014, *6*, 1270–1276.

[4] M. Frese, N. Sewald, *Angew. Chem. Int. Ed.* 2015, *54*, 298–301.
[5] M. Frese, C. Schnepel, H. Minges, H. Voß, R. Feiner, N. Sewald, *ChemCatChem* 2016, *8*, 1799–1803.
[6] C. Schnepel, H. Minges, M. Frese, N. Sewald, *Angew. Chem. Int. Ed.* 2016, *55*, 14159–14163.

Presentation: Monday, 6 March 2017 from 19:00 - 19:30 in room 10-11.

MINISYMPOSIA 12
Phenotyping in the genomic age (FG IS)
06 March 2017 • 18:00 – 19:30

075/ISV
In-silico Chemotaxonomy: A tool for 21st century microbial systematics
P. Lawson*[1], K. Sankaranayanan[1], N. Patel[1], H.-J. Busse[2]
[1]University of Oklahoma, Microbiology and Plant Biology, Norman, Oklahoma, United States
[2]University of Wien, Microbiology and Genetics Institute, Vienna, Austria

Currently, the focus of a robust debate between microbiologists is the very concepts and methods used in prokaryotic systematics and how this should be approached in the future [1]. Opinions range from the use of a polyphasic approach in tandem with phylogenetic analyses, to utilizing only the genome with a few minimal phenotypic traits being described [2, 3]. A unifying theme emerges whereby a strong taxonomy must encompass sufficient biological markers to make the assignment of taxa to a particular group a robust process. Information from genomic analysis derived via affordable next generation sequencing must be embraced, but not at the expense of good taxonomic practices. In addition to being labor intensive and impacted by a lack of cross-laboratory standardization, chemotaxonomic methods also suffer from a lack of expertise and searchable databases to retrieve comparative data. The ease of generating whole genome sequences now makes it possible to utilize metabolic pathway information and genome annotation as an alternative tool to examine these important diagnostic biomarkers. Bacterial strains distributed across five phyla, with published genomes will be used as a reference to develop "*in silico"* prediction models. The phyla targeted in this study comprise microbes with clinical, environmental, and broader biotechnological relevance. Genomic data will be compared with published data for chemotaxonomic features; where necessary data and characterization of unknown markers will be generated in-house. Once prediction models are built, they will be evaluated on a separate set of organisms spanning a similar phylogenetic range. The development of these models and tools (searchable, curated web-based databases) have great potential to revolutionize chemotaxonomy making it accessible to the broader scientific community rather than the relatively small number of specialist laboratory that currently undertake this analysis.

References
[1] Sutcliffe IC, Trujillo ME, Goodfellow M. (2012). The BISMiS 2011 special issue on prokaryotic systematics, a vital discipline entering a period of transition. *Antonie van Leeuwenhoek* 10:1-2.
[2] Oren A. (2004). Prokaryote diversity and taxonomy: current status and future challenges. *Philosophical Transactions of the Royal Society B: Biological Sciences* 359: 623-38.
[3] Whitman WB (2014). The Need for Change: Embracing the Genome. *Methods in Microbiology* 41: 1-12.

Presentation: Monday, 6 March 2017 from 18:00 - 18:30 in room 5.

076/ISV
The role of the phenotype in the systematics of prokaryotes
P. Kaempfer*[1]
[1]Justus-Liebig-University, Institute for Applied Microbiology, Giessen, Germany

Systematics can be considered as a comprehensive science, because in science it is an essential aspect in comparing any two or more elements, whether they are genes or genomes, proteins or proteomes, biochemical pathways or metabolomes (just to list a few examples), or whole organisms. The development of high throughput sequencing techniques has led to an enormous amount of data (genomic and other “omic” data) and has also revealed an extensive diversity behind these data.
These data are more and more used also in systematics and there is a strong trend to classify and name the taxonomic units in prokaryotic systematics preferably on the basis of sequence data. Unfortunately, the knowledge of the meaning behind the sequence data does not keep up with the tremendous increase of generated sequences.
Traditionally the polyphasic approach in bacterial systematics considers methods including both phenotype and genotype. The criteria used for systematics may change when we have a full insight into the complexity of the genomes (and the "phenome”) of microorganisms. However, phenotypic characters can contribute useful and essential informations in modern systematics.
And it is the phenotype that is (also) playing an essential role in driving the evolution.

Presentation: Monday, 6 March 2017 from 18:30 - 19:00 in room 5.

077/ISV
Mass Spectrometry Proteotyping: Proteomics- and Genomics-Based Characterisation and Diagnostics of Infectious Bacteria
E. R. B. Moore*[1,2], L. Gonzales-Siles[1], H. E. Jakobsson[1], F. Salvà-Serra[1,3], M. Gomila[3], A. Busquets[3], A. Bennasar[3], S. Skovbjerg[2], F. Boulund[4], E. Kristiansson[4], R. Karlsson[2,5]
[1]University of Gothenburg, Department of Infectious Diseases, Gothenburg, Sweden
[2]Sahlgrenska University Hospital, Department of Clinical Microbiology, Gothenburg, Sweden
[3]University of the Balearic Islands, Department of Biological Sciences, Palma de Mallorca, Spain
[4]Chalmers University of Technology, Department of Mathematical Sciences, Gothenburg, Sweden
[5]Nanoxis Consulting AB, Gothenburg, Sweden

The global expansion of anti-microbial resistance (AMR) in bacteria, including human pathogens, presents difficult challenges for treatment and the prevention of the spread of infection. The World Health Organisation (WHO) has predicted the advent of infectious diseases for which no antibiotic treatment will be available [1]. With such escalation of AMR, combined with continuing decline in new antibiotic discovery, development of innovative, reliable, rapid and cost-efficient analytical techniques for effective characterisations and diagnostics of infectious microorganisms is increasingly essential to confront rising mortality and costs associated with AMR infections. However, the routine methodologies used today for diagnosing infectious disease in most cases depend upon protocols requiring prior cultivation of pathogenic bacteria from clinical samples. Faced with patients exhibiting symptoms of severe infection, physicians often resort to prescribing broad-spectrum antibiotics while they may wait days or weeks for results from the laboratory.
With increasing whole-genome sequence data becoming available, MS-based proteomics also have increasingly been applied to biological studies. Proteomic analyses of bacterial cells may be considered to be indirect analyses of the genomes. The proteome comprises the entire set of proteins expressed by a cell, an

organism or a biological system. Proteotyping [2], using state-of-the-art LC-MS/MS analyses of generated cellular peptides, enables identification of the most closely related bacterial species, as well as sub-species-level discrimination, AMR- and virulence-factors, from single MS analyses. Comprehensive and accurate genome sequence data is a key to obtaining accurate peptide matching and to be able to discriminate the most closely related species. In this study, genome sequences were analysed, using Average Nucleotide Identity based on Blast (ANIb), core genome and taxon-specific MLSA to assess systematic reliabilities. Critically, significant numbers of sequenced genomes in the public databases exhibited questionable and incorrect identifications.
Characterisations and identifications of responsible agents of infectious disease have relied heavily upon established systematic frameworks and the documented features of well-described microbial taxa. As new methodologies, such as genomics and proteomics are developed to enable more comprehensive, detailed and complex analyses, comprehensive databases linked with a reliable systematic framework are essential for reliable diagnostics.

References
[1] World Health Organization. 2014. Antimicrobial Resistance: Global Report on Surveillance. ISBN: 978-92-4-15674-8.
[2] Karlsson et al. 2015. Proteotyping: Proteomic characterization, classification and identification of microorganisms – A prospectus. Syst Appl Microbiol 38:246-257.

Presentation: Monday, 6 March 2017 from 19:00 - 19:30 in room 5.

MINISYMPOSIA 13
Chromosome organisation and dynamics - from 3D structures to synthetic replicons (FG MZ)
06 March 2017 • 18:00 – 20:00

078/MZV
How SMC condensin complexes compact and resolve replicated chromosomes (What Bacillus subtilis tells us)
D. Rudner*[1], X. Wang[1], H. Brandao[1]
[1]*Harvard Medical School, Microbiology, Boston, Massachusetts, United States*

SMC complexes play critical roles in chromosome dynamics in virtually all organisms but how they function remains poorly understood. In *Bacillus subtilis*, SMC condensin complexes are topologically loaded at centromeric sites adjacent to the replication origin. Here we provide evidence that these ring-shaped assemblies tether the left and right chromosome arms together as they travel from the origin to the terminus (>2 Mb) at rates >50kb/min. Globally, condensin movement scales linearly with time arguing for an active transport mechanism. Locally, movement is influenced by DNA transactions encountered along its path. Collectively, our data support a model in which SMC complexes function by processively enlarging DNA loops. De novo loop formation by processive loop enlargement provides a mechanism for how Eukaryotic condensin complexes resolve sister chromatids in mitosis and how SMC cohesin complexes generate topologically associating domains (TADs) during interphase.

Presentation: Monday, 6 March 2017 from 18:00 - 18:30 in room 12.

079/MZV
Structural maintenance of chromosomes: In the arms of SMC proteins
F. Bürmann*[1], S. Gruber[1]
[1]*Max Planck Institute of Biochemistry, Martinsried, Germany*

The chromosome is the primary carrier of genetic information in bacteria. Thus, mechanisms that make chromosome inheritance reliable have a large impact on population fitness. Ensuring the complete and timely segregation of replicated chromosomes to daughter cells, however, appears non-trivial: The chromosome is a single DNA molecule with a contour length of a few millimeters, and it is packaged inside a cell that is about a thousand times shorter. In consequence chromosomal DNA is likely prone to entanglement. Chromosome segregation is promoted by structural maintenance of chromosomes (SMC) proteins, which possibly resolve entanglements through the extrusion of DNA loops. SMC proteins are ATPases with a large coiled-coil "arm", but if and how the arm is important for their biological activity is largely unknown. Here we present evidence that the coiled-coil arm is intimately involved in SMC function, in a manner that strongly depends on its super-helical structure and mechanical integrity. It appears that the arm couples SMC ATPase activity to a directed movement on the chromosome.

Presentation: Monday, 6 March 2017 from 18:30 - 19:00 in room 12.

080/MZV
A new bacterial replication origin element specifies single-strand initiator binding
T. Richardson[1], O. Harran[1], H. Murray*[1]
[1]*Newcastle University, Centre for Bacterial Cell Biology, Newcastle upon Tyne, United Kingdom*

DNA synthesis is an essential activity for cell proliferation. In all organisms a conserved family of AAA+ proteins initiates DNA replication at chromosome origins. There are major gaps regarding our understanding of how chromosome origins direct initiator protein activity. Recently we reported the identification of a new bacterial chromosome origin element, the DnaA-trio, in the model organism *Bacillus subtilis* (Richardson, Harran, and Murray. *Nature* 2016). We found that DnaA-trios are essential for origin function and that they guide filament assembly of the master bacterial initiator protein DnaA onto a single DNA strand. In this talk I will discuss both the discovery of the DnaA-trio and our ongoing work to characterize its structure and function.

Presentation: Monday, 6 March 2017 from 19:00 - 19:30 in room 12.

081/MZV
Synthetic secondary chromosomes to study chromosome maintenance in *Escherichia coli*
T. Waldminghaus*[1]
[1]*Philipps-Universität, SYNMIKRO, Marburg, Germany*

Chromosomes need systems to replicate, segregate and organize the encoded genetic information. Many such chromosome maintenance systems consist of proteins binding to DNA motifs with specific chromosomal distributions. Examples in *E. coli* include the nucleoid occlusion mediated by SlmA or the MatP protein that organizes the Ter macrodomain. While many studies focused on the proteins and binding to individual DNA motifs much less is known about the role of the genomic motif distribution. One reason is the lack of suitable experimental approaches. We want to fill this gap by constructing synthetic secondary chromosomes of about 100 kbp with variation of DNA-motif distributions. Downstream functional characterization should allow deeper understanding of chromosome maintenance. Our main focus is on the epigenetic GATC system that regulates

DNA mismatch repair, chromosome segregation and DNA replication in *E. coli* and related bacteria. As first step, we have constructed and characterized the synthetic secondary chromosome backbone synVicII based on the replication origin of the natural secondary chromosome of *Vibrio cholerae*. This backbone was extended towards three larger chromosomes (~100kbp) with different GATC distributions. Measuring respective mutation rates is ongoing and should uncover what type of functional interaction connects the mismatch repair mediated by MutH and SeqA-dependent chromosome segregation.

Presentation: Monday, 6 March 2017 from 19:30 - 20:00 in room 12.

MINISYMPOSIA 14
Fungi, in silico! (FG FB)
06 March 2017 • 18:00 – 20:00

082/FBV

Patterns of genomic variation in the opportunistic pathogen *Candida glabrata* suggest the existence of mating and a secondary association to the human host

L. Carreté[1,2], E. Ksiezopolska[1,2], C. Pegueroles[1,2], E. Gómez-Molero[3], E. Saus[1,2], S. Iraola-Guzmán[1,2], D. Loska[1,2], O. Bader[3], C. Fairhead[4], T. Gabaldón[1,2,5*]

[1] *Bioinformatics and Genomics Programme. Centre for Genomic Regulation (CRG), the Barcelona Institute of Science and Technology (BIST). Dr. Aiguader, 88. Barcelona, Spain.*

[2] *Universitat Pompeu Fabra (UPF). Barcelona, Spain.*

[3] *Institute for Medical Microbiology, University Medical Center Göttingen, Göttingen, Germany.*

[4] *GQE– Le Moulon, INRA – Université Paris-Sud – CNRS – AgroParisTech, Orsay, France.*

[5] *Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluís Companys 23, Barcelona, Spain.*

Background: *Candida glabrata* is an opportunistic fungal pathogen that ranks as the second most common cause of systemic candidiasis. *C. glabrata* has been considered to be asexual and its ability to infect humans is thought to have emerged only recently during evolution.

Results: We assessed genomic and phenotypic variation across 33 globally-distributed *C. glabrata* isolates. We cataloged extensive copy number variation, which particularly affects genes encoding cell-wall associated proteins, including adhesins. The level of genetic variation in *C. glabrata* is significantly larger than that found in *Candida albicans*. We found seven deeply divergent clades, which show recent geographical dispersion and large within-clade genomic and phenotypic differences. We show compelling evidence of recent admixture, and of purifying selection on mating genes.

Conclusions: Altogether, these findings support the existence of a sexual cycle, and suggest that humans are only

Presentation: Monday, 6 March 2017 from 18:00 - 18:30 in room 7-8.

083/FBV

Surface-exposed proteome of the opportunistic fungal pathogen *Aspergillus fumigatus*

M. Blango*[1], T. Krüger[1], M. Röcker[1], O. Kniemeyer[1], A. Brakhage[1]

[1]*Hans Knöll Institute, Molecular and Applied Microbiology, Jena, Germany*

Introduction: *Aspergillus fumigatus* is a soil saprophyte, opportunistic fungal pathogen, and major source of allergic respiratory disease in humans. Although invasive aspergillosis can be deadly in immunocompromised patients, it is more commonly observed as a complication of respiratory diseases like asthma and chronic obstructive pulmonary disease, presenting as allergic bronchopulmonary aspergillosis (ABPA) or severe asthma with fungal sensitization (SAFS). ABPA and SAFS encompass a wide range of severity and affect millions of asthmatics annually [1]. Fungal conidia that have started germination (swollen conidia) are the likely causative agents of allergic exacerbation, as dormant conidia are biologically inert [2].

Objectives: The objectives of this study are to elucidate the surface-exposed proteome of dormant and germinating *A. fumigatus* conidia, identify proteins that contribute to exacerbation of allergic disease, and characterize proteins of interest using recombinant protein technologies in conjunction with cell culture and *in vivo* model systems.

Materials & methods: Surface-exposed proteins from dormant and germinating conidia were proteolytically cleaved by brief incubation with trypsin, followed by LC-MS/MS analysis. In addition, secreted proteins were collected from the supernatant of swollen conidia and enriched by phase separation before identification with LC-MS/MS.

Results: We identified 177 unique surface-exposed proteins across four stages of germination (dormant conidia 55, swollen conidia 140, germlings 56, and hyphae 109) with the hydrophobin RodA being the most abundant. ~20% of proteins were common at all four time points, whereas swollen conidia and hyphae exhibited the largest set of stage-specific proteins, 49 and 30 proteins, respectively. Many of the identified proteins are in GO slim categories for hydrolase activity, protein binding, and oxidoreductase activity, with another large fraction having no known molecular function. From the supernatant of swollen conidia we observed only three proteins: Ecm33, a GPI-anchored protein; Bgt2, a cell wall glucanase; and a small secreted peptide with no known function and no sequence homology outside of *Aspergillus*.

Conclusion: Although preliminary, we have identified multiple proteins of interest from germinating conidia. In future experiments we aim to characterize some of these proteins for the capacity to trigger allergic exacerbation in cell culture and mouse models. In addition, we hope to elucidate the surface-exposed proteomes of other common allergenic fungal species, including *Penicillium chrysogenum*, *Alternaria alternata*, and *Cladosporium herbarum*. Ultimately we hope to identify novel protein targets that contribute to fungal allergy.

References

[1] Denning DW, *et al*. 2013. *Medical Mycology* 51(4):361-370.

[2] Chaudhary N, *et al.* 2011. *Clinical and Translational Allergy* 1(4)

Presentation: Monday, 6 March 2017 from 18:30 - 18:45 in room 7-8.

084/FBV

Genomic variations in the human pathogenic fungus Lichtheimia

V. Schwartze*[1,2], J. Linde[3], M. Nowrousian[4], S. Roos[1,2], M. Groth[5], T. Veselská[6], I. D. Jacobsen[7], U. Binder[8], O. Kniemeyer[9], K. Voigt[1,2]
[1]*Friedrich-Schiller-University Jena, JMRC, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, JMRC, Jena, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Systems Biology and Informatics, Jena, Germany*
[4]*Ruhr University Bochum, Department of General and Molecular Botany, Bochum, Germany*
[5]*Leibniz Institute on Aging – Fritz Lipmann Institute, Jena, Germany*
[6]*Charles University, Department of Botany, Prague, Czech Republic*
[7]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Microbial Immunology, Jena, Germany*
[8]*Medical University Innsbruck, Division of Hygiene and Medical Microbiology, Innsbruck, Austria*
[9]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Molecular and Applied Microbiology, Jena, Germany*

Members of the order Mucorales belong to the most basal terrestrial fungi. Most of the species are saprotrophic organisms but some species can also cause life-threatening infections in humans, called mucormycosis. While mucormycoses are uncommon infections, they have been increasingly recognized during the last decades. Despite their clinical importance, only little attention has been paid to mucoralean fungi and the molecular pathogenicity mechanisms are largely unknown. Members of the genus *Lichtheimia* represent the second-most common cause of mucormycosis in Europe.
To gain further insights into *Lichtheimia* species, the genomes of three strains of the human pathogens *L. corymbifera* and *L. ramosa* were sequenced and analysed. All genomes were characterised by the presence of extensive gene duplications, which are due to a combination of an ancient whole genome duplication event and additional single gene duplications. Most of the analysed genomes were highly similar and showed only minor differences in their genome architecture. Like all other mucoralean fungi investigated so far, *Lichtheimia* species grow as homokaryons with haploid nuclei. However, one isolate showed differences in the genome size and appears to be a heterokaryon. The generation of single spore isolates from this strain resulted in the availability of homokaryotic strains with distinct phenotypes and reduced virulence.
The data of this study give first insights into the variability of the genome architecture in basal mucoralean fungi. In addition, the availability and analysis of isolates with reduced virulence will contribute to the identification of virulence-associated traits in these human pathogens.

Presentation: Monday, 6 March 2017 from 18:45 - 19:00 in room 7-8.

085/FBV

RNA-seq and ChIP-seq reveal control mechanisms in fungal sexual development

I. Teichert*[1], E. K. Steffens[1], T. A. Dahlmann[1], M. Nowrousian[1], U. Kück[1]
[1]*Ruhr-Universität Bochum, Lehrstuhl für Allgemeine und Molekulare Botanik, Bochum, Germany*

Introduction: Filamentous fungi are ubiquitous organisms that can be both deleterious and highly beneficial. The understanding of fungal biology is a fundamental prerequisite for industrial and pharmaceutical applications. The filamentous fungus *Sordaria macrospora* has a long history as a model system for studying fungal biology, especially sexual development. Characterization of sterile mutants led to the identification of many developmental factors required for fruiting body formation. These factors include phosphatases, kinases, signaling protein, transcription factors (e.g. PRO1), NADPH oxidases (e.g. NOX1), and autophagy proteins [1, 2]. However, how these developmental factors are orchestrated is just beginning to emerge.
Objectives: We set out to identify new regulators of fungal sexual development and to gain insight into conserved mechanisms in fungal biology by functional genomics analysis.
Materials and Methods: RNA-seq with mutants pro1 and nox1 as well as ChIP-seq studies with PRO1 served as basis for the identification of regulators and regulatory systems involved in sexual development.
Results: ChIP-seq analysis and EMSA studies revealed that PRO1 binds to promoters of known signaling proteins involved in sexual development in *S. macrospora*. These proteins include members of the pheromone and cell wall integrity MAP kinase pathways, and NADPH oxidase complexes. Regulation was verified at the transcript level in RNA-seq and Real-time PCR, showing that PRO1 is a master regulator of fungal sexual development [3]. Further analysis of RNA-seq data from mutant and wild type sexual structures revealed the occurrence of RNA editing in wild type fruiting bodies, but not mutants pro1 and nox1. Editing events are found within coding sequences and lead to changes in amino acid sequence of the encoded proteins.
Conclusion: We identified regulatory mechanisms for fungal sexual development at different levels of gene expression. While transcription factors like the master regulator PRO1 control gene expression at the transcriptional level, further factors control expression at the post-trancriptional level. Both mechanisms are required for efficient fruiting body formation.

References

[1] Teichert et al. 2014 Adv Genet 87:199
[2] Teichert et al. 2014 PLoS Genet 10:e1004582
[2] Steffens et al. 2016 Mol Microbiol, in press

Presentation: Monday, 6 March 2017 from 19:00 - 19:15 in room 7-8.

086/FBV

The identification and role of ncRNAs during fungal infections

K. Riege*[1], M. Marz[1]
[1]*Frierich-Schiller-University Jena, RNA Bioinformatics and High Throughput Analysis, Jena, Germany*

The prediction of protein coding genes and non-(protein-)coding RNAs (ncRNAs) in genomic sequencing data is a challenging task in investigation of biological processes. NcRNAs play essential roles in regulating several basic cellular processes, such as transcription, processing, and translation of protein-coding genes. About 80% of the human genome is supposed to have a function, but less then 3% code for proteins [1]. This calls for the identification of to date unknown ncRNAs. Due to the high variability and structural properties of many ncRNA families, no universal homology based strategy is existing so far. We present GORAP - a genome-wide functional ncRNA annotation pipeline for all 2468 RNA families provided by the Rfam database [2]. This software suite combines established standard and advanced tools extended by taxonomic and family specific filters to obtain low false positive prediction rates. GORAP"s modular design and high configurability allows to easily plug in new software and query RNA models. Applied on different genome studies [3,4,5], GORAP completes the picture of ncRNA landscape exploration by RNA-Seq driven *de novo* gene prediction and expression analysis.
NcRNAs as transcription factors or cis acting antisense RNAs are known to be involved in gene regulation. We investigated the effects during Mycoses induced by the pathogenic fungi *C. albicans* or *A. fumigatus*, leading to important host damage either by deficient or exaggerated immune response [6]. The regulation

of chemokine and cytokine signaling plays an crucial role for an adequate inflammation, which can be modulated by vitamins A and D [7,8]. We studied the treatment with vitamins in terms of therapeutic impact and show that in monocytes both vitamins regulate ncRNAs involved in amino acid metabolism and immune system processes using comprehensive RNA-Seq analyses. Compared to proteins less ncRNAs were differentially expressed, but with a massive fold change of up to 4000, from which most of the involved antisense RNAs are regulated and positively correlated with their sense coding genes. Long-ncRNAs with stimulus specific immunomodulatory activity were identified as potential marker genes for a possible detection in the early phase of fungal infection and as potential therapeutic targets in the future.

References

[1] ENCODE Project Consortium. An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57–74 (2012).
[2] Nawrocki, E P et al. Rfam 12.0: updates to the RNA families database. *Nucleic Acids Res* 43, D130–D137 (2015).
[3] Hölzer, M et al. Differential transcriptional responses to Ebola and Marburg virus infection in bat and human cells. *Sci Rep* 6, (2016)
[4] Linde, J et al. Defining the transcriptomic landscape of Candida glabrata by RNA-Seq. *Nucleic Acids Res* 43, 1392-1406 (2015)
[5] Lechner, M et al. Genomewide comparison and novel ncRNAs of Aquificales. *BMC Genomics* 15, 522-522 (2014)
[6] Romani L. Immunity to fungal infections. *Nat Rev Immunol* 4, 11-24 (2004)
[7] Hall, J A et al. The role of retinoic acid in tolerance and immunity. *Immunity* 35, 13-22 (2011)
[8] Prietl, B et al. Vitamin d and immune function. *Nutrients* 5, 2502-2521 (2013)

Presentation: Monday, 6 March 2017 from 19:15 - 19:30 in room 7-8.

087/FBV

An *in-silico* reconstructed gene regulation network for *Aspergillus niger* for the prediction of protein function

N. Paege*[1], S. Jung[1], P. Schäpe[1], V. Meyer[1]
[1]*TU Berlin, Applied and Molecular Microbiology, Berlin, Germany*

The genome of *Aspergillus niger* belongs to the best annotated genomes among *Aspergillus* species; however, only 2% of its ~14,000 genes are functionally verified and 50% of the predicted open reading frames encode for hypothetical proteins. Hence, the genetic basis for almost all cellular processes in *A. niger* and its physiological peculiarities is unknown. On the other hand, hundreds of post-genomic data including transcriptomic and proteomic data are available for *A. niger* for more than 150 different growth conditions. This holistic dataset can be scrutinized and used to predict gene functions and gene interactions in *A. niger*. For a proof-of-concept, we have focused on the *anafp* gene encoding the antifungal protein AnAFP known to selectively inhibit the growth of filamentous fungi. As more than 50 AnAFP orthologs have been identified in many different genera of the *Ascomycota* tree of life, we wished to understand which regulatory systems control expression of the *anafp* gene.
We have performed a meta-analysis of an in-house transcriptome database harboring genome-wide expression data for more than 150 growth conditions of *A. niger*. This analysis uncovered that *anafp* displays a highly coordinated temporal and spatial transcriptional profile which is concomitant with key nutritional and developmental processes. Its expression profile coincides with early starvation response and parallels with genes involved in nutrient mobilization and autophagy. Using fluorescence- and luciferase reporter strains we could demonstrate that the *anafp* promoter is indeed under control of CreA and FlbA as predicted by the *in silico* data and highly expressed in highly in vacuolated compartments and foraging hyphal cells during carbon starvation. A co-expression network analysis further predicted that *anafp* expression is embedded in several cellular processes including allorecognition, osmotic and oxidative stress survival, development, secondary metabolism and autophagy, and predicted StuA and VelC as additional regulators (1). We currently prove these predictions by respective wet-lab experiments. Our work illustrates how *in silico* transcriptomic analysis can lead to hypotheses regarding protein regulation, protein function and its embedding in different biological processes.

Presentation: Monday, 6 March 2017 from 19:30 - 19:45 in room 7-8.

088/FBV

Genetic barcoding and metabarcoding in microbiology

A. Salawu-Rotimi*[1], A. M. Rotimi[2], O. N. Reva[2]
[1]*University of Pretoria, Biochemistry(Bioinformatics), Pretoria, South Africa*
[2] *University of Pretoria, Department of Biochemistry, Bioinformatics and Computational Biology Unit, Pretoria, South Africa*

A wide range of genetic data about organisms of interest has become obtainable with the advancement to next generation sequencing (NGS). However, it remains a challenge for researchers to process this enormous volume of genetic data released by NGS and to apply this information to resolve practical questions. Genetic barcoding and metabarcoding of microorganisms is the first obvious area where NGS has met the need of applied microbiology.The aim of this study was to develop an algorithm for effective design of diagnostic barcodes based on multiple signature genomic loci to distinguish between closely related microorganisms. In this study genetic barcode sequences were created for several taxonomic groups of microorganisms of medical and biotechnological importance. The developed barcodes were tested on metagenomics datasets representing different echo-niches. Sets of metagenomic reads were obtained from NCBI and MG-RAST databases. The DNA reads were aligned against the barcode sequence by BLASTN using an in-house Python script. In addition to getting information regarding the natural distribution of different microorganisms, this program evaluated selectivity and specificity of fragments of the barcode sequences to allow noise reducing and improvement of sensitivity of the method. Statistical approaches of barcode evaluation were considered. The developed python script returned also rich graphical representations of the results of identification of species of interest in different habitats.The development of this computer based system will aid in tracking down biotechnological strains in the environment. The algorithm will also be beneficial in medicinal bacteriology for tracing down virulent microorganisms and causative agents of disease outbreaks.

Presentation: Monday, 6 March 2017 from 19:45 - 20:00 in room 7-8.

MINISYMPOSIA 15
Regulation of CO2 fixation and carbon metabolism in cyanobacteria (FG CB)
06 March 2017 • 18:00 – 20:00

089/CBV
Viral photosynthesis: Rewiring the cyanobacterial photosynthetic factory
R. Puxty*[1], A. Millard[1], D. Evans[1], D. Scanlan[1]
[1]*University of Warwick, Life Sciences, Coventry, United Kingdom*

The cyanobacterial genera *Prochlorococcus* and *Synechococcus* are responsible for approximately 10% of biospheric photosynthesis. Viruses of these genera (cyanophage) are exceptional in that they frequently contain genes thought to be involved in photosynthesis. It was therefore posited that cyanophage play a direct role in photosynthesis- so called viral photosynthesis. Our data suggest cyanophage infected cells have increased photosynthetic electron transport (PET) under higher irradiance compared with uninfected cells. Further, such high light infections result in decreased latency periods such that cyanophage productivity is increased. Meanwhile cyanophages actively shut down host CO2 fixation whilst maintaining PET. Thus our combined evidence from comparative genomic, physiological and transcriptomic experiments suggest a nuanced mode of action of viral photosynthesis during infection: One that starves the host cell of carbon in exchange for the production of energy for morphogenesis of viral particles. We further speculate on the functioning of specific viral genes responsible for this rewiring that are currently under investigation.

Presentation: Monday, 6 March 2017 from 18:00 - 18:30 in room 6.

090/CBV
Re-engineering carbon fixation: challenges, benefits, and vision
A. Bar-Even*[1,2]
[1]*Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany*
[2]*Research Institute, Plant Sciences, Potsdam-Golm, Germany*

Carbon fixation is one of pillars of cellular metabolism, directly responsible for integrating the most important element of life into the biosphere. While the Calvin Benson Cycle is responsible for most of the assimilated CO2, other carbon fixation routes are known to operate in various prokaryotic lineages. As there are at least 20 known carboxylating enzymes, spread throughout the global metabolic network, it is possible to envision a wild array of synthetic pathways, designed by integrating existing enzymes into novel structures. Indeed, multiple such synthetic alternative carbon fixation pathways were suggested in recent years. Very recently, one such pathway, the CETCH cycle, was established *in vitro*, and was found to support higher carbon fixation than the Calvin Benson Cycle.
While the implementation of synthetic pathways might support higher carbon fixation rate and/or higher growth yield, it involves numerous challenges. Most important of those are the integration of the new pathway within the endogenous metabolic network and the establishment of suitable regulatory system for the new route. Furthermore, to fully understand the advantages and drawbacks of each candidate route, an intricate methodology of pathway analysis must be employed. Specifically, several physiochemical properties must be addressed, including thermodynamic feasibility and driving force, kinetic proficiency and bottlenecks, hydrophobicity and reactivity of intermediates, and dependence on environmental conditions such as concentrations fo O2 and CO2. As different applications require different sets of properties, the choice of the most suitable synthetic pathway will change accordingly.
An interesting and potentially highly promising approach to sustain efficient carbon fixation involves the reduction of CO2 to formate, followed by formate assimilation into central metabolism. While this approach is limited to conditions where low reduction potential electron donors are available to support CO2 reduction, it can support the assimilation of CO2 at a minimal ATP cost, minimal number of reactions, and minimal overlap with central metabolism. Hence, formate-mediated carbon assimilation might provide especially efficient solutions for various future needs.

Presentation: Monday, 6 March 2017 from 18:30 - 19:00 in room 6.

091/CBV
The physiology of the Entner-Doudoroff pathway in cyanobacteria
A. Makowka[1], B. Fähnrich[1], K. Spengler[1], K. Gutekunst*[1]
[1]*Christian-Albrechts-Universität, Botanisches Institut, Kiel, Germany*

Photoautotrophs fuel their metabolism in a circuit of photosynthesis and carbohydrate oxidation. Carbohydrates were long known to be broken down via the oxidative pentose phosphate (OPP) patwhay and the Emden-Meyerhof-Parnas (EMP) pathway, often referred to as glycolysis (1,2). We could recently show that a third previously overlooked glycolytic route, the Entner-Doudoroff (ED) pathway, operates in both cyanocacteria and plants (3). The pathway was transferred via endosymbiotic gene transfer from cyanobacteria to the plant kingdom. In comparison to the EMP patwhay, the ED pathway is much more prevalent in cyanobacteria. In order to evaluate the physiological significance of OPP-, EMP-, and ED pathway, mutants in which different glycolytic routes were interrupted were characterized under multiple conditions. These measurements reveal that the ED pathway might be especially important in connection with photosynthesis. The interplay of glycolytic routes, CO_2-fixation and photosynthesis will be discussed.

References
[1] Knowles, V. L., and Plaxton, W. C. (2003) From Genome to Enzyme: Analysis of Key Glycolytic and Oxidative Pentose-Phosphate Pathway Enzymes in the Cyanobacterium Synechocystis sp. PCC 6803. *Plant and Cell Physiology* 44, 758-763
[2] Plaxton, W. C., and Podesta, F. E. (2006) The functional organization and control of plant respiration. *Critical Reviews in Plant Sciences* 25, 159-198
[3] Chen, X., Schreiber, K., Appel, J., Makowka, A**.,** Fähnrich, B., Roettger, M., Hajirezaei, M. R., Sönnichsen, F. D., Schönheit, P., Martin, W. F., and Gutekunst, K. (2016) The Entner–Doudoroff pathway is an overlooked glycolytic route in cyanobacteria and plants. *Proceedings of the National Academy of Sciences* 113, 5441-5446

Presentation: Monday, 6 March 2017 from 19:00 - 19:15 in room 6.

092/CBV

Glycogen catabolism is essential for resuscitation of chlorotic cyanobacteria

A. Klotz*[1], J. Georg[2], W. R. Hess[2], K. Forchhammer[1]
[1]*University of Tuebingen, IMIT - Organismische Interaktionen, Tuebingen, Germany*
[2]*University of Freiburg, Faculty of Biology - Genetics and Experimental Bioinformatics, Freiburg, Germany*

Glycogen synthesis is essential for cyanobacteria to cope with stress and to maintain viability during longer periods of darkness [1]. Furthermore, mutants deficient in glycogen synthesis are not able to adapt to nitrogen depletion display a non-bleaching phenotype and die during starvation [1]. During nitrogen starvation *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) produces reserve biopolymers like glycogen and polyhydroxybutyrate and is able to maintain viable for a long period of time due to low-level photosynthesis.
In our study, the resuscitation of chlorotic *Synechocystis* cells from long-term chlorosis was used as a model to study the awakening of dormant cells [2]. The re-greening process was highly reproducibility and synchronous. A detailed molecular analysis revealed a highly orchestrated and genetically determined resuscitation program. In the first phase of this process the cells switched off the residual photosynthesis despite illumination. They turned on respiration and consumed glycogen thereby overcoming the Kok effect. As determined by transcriptome analysis, the expression of genes required for glycogen degradation showed a remarkable reverse dynamics compared to glycogen consumption. Instead of being induced, these genes are moderately repressed. In fact, the cells seem to anticipate and prepare for glycogen degradation already during synthesis of the polymer. Phase two is defined by the switch to photosynthetic metabolism and the re-greening of the cells. Interestingly, the cells continue to degrade glycogen despite active photosynthetic oxygen evolution. To further understand the role of glycogen and the mobilization of glycogen during resuscitations mutants deficient in the glycogen phosphorylase, degrading glycogen to glucose-1-P, were created and analyzed. These mutants show impaired resuscitation and are unable to degrade glycogen.
Our case study of resuscitation of dormant *Synechocystis* cells demonstrated that this process follows a genetically determined program and that the cells act anticipatory [2]. Moreover, glycogen plays a major role during stress adaptation as well as stress relief in cyanobacteria. Glycogen metabolism and its interconnections are of fundamental importance herein.

References

[1] Gründel, M., Scheunemann, R., Lockau, W., and Zilliges, Y. (2012). Impaired glycogen synthesis causes metabolic overflow reactions and affects stress responses in the cyanobacterium *Synechocystis* sp. PCC 6803. Microbiology *158*, 3032-3043.
[2] Klotz, A., Georg, J., Bučinská, L., Watanabe, S., Reimann, V., Januszewski, W., Sobotka, R., Jendrossek, D., Hess, Wolfgang R., and Forchhammer, K. (2016). Awakening of a Dormant Cyanobacterium from Nitrogen Chlorosis Reveals a Genetically Determined Program. Current Biology *26*, 2862-2872.

Presentation: Monday, 6 March 2017 from 19:15 - 19:30 in room 6.

093/CBV

Do sustained differences in CO2 availability affect net photosynthesis and toxin production in diazotrophic Cyanobacteria?

M. Gehringer*[1], C. Colesie[1], N. Wannicke[1], B. Büdel[1]
[1]*TU Kaiserslautern, Plant Ecology & Systematics, Kaiserslautern, Germany*

Increasing levels of CO2 in the atmosphere are suggested to favour increased incidences of cyanobacterial blooms in marine and freshwater environments. Nitrogen fixing (diazotrophic) cyanobacteria are capable of forming large blooms without nutrient enrichment (eutrophication) of water masses, often producing toxins. The process of oxidative photosynthesis by Cyanobacteria evolved under conditions of elevated CO2 (eCO2) during the Archean era ranging from 5-10× that of present atmospheric levels (PAL). The aim of this pilot study was to investigate whether eCO2, as predicted by climate change models, leads to increased net photosynthesis (NP) rates in diazotrophic cyanobacterial species from both aquatic and terrestrial environments. Both toxin and non-toxin producing strains of *Nostoc* and *Nodularia* were grown at PAL (control) or eCO2 (treatment, 5× PAL) and NP measured at PAL (ambient) and 1000 ppm CO2 (high). Control cultures were immediately able to increase NP when measured at high CO2 levels, whereas cultures accustomed to growing at eCO2 exhibited reduced NP at ambient CO2 levels. There was no direct correlation between increased nodularin production and eCO2 in either aquatic, or terrestrial nodularin producing species. NP was significantly higher in the aquatic *Nodularia* species than the terrestrial *Nostoc* species tested. This study has demonstrated that growth under eCO2 is energetically favourable for Cyanobacterial species, as they are not reliant on the energy demanding processes of CCM and photorespiration to ensure efficient NP and detoxification under modern-day oxygen rich, CO2 reduced conditions. The variation in species response could be attributed to evolutionary status and adaptation to modern-day atmospheric conditions and potential genome streamlining. Future studies should be undertaken with cyanobacterial strains for which genomic sequence data is available to better investigate specifics relating to NP and CO2 availibility.

Presentation: Monday, 6 March 2017 from 19:30 - 19:45 in room 6.

094/CBV

A small RNA which contributes to the regulation of central enzymes in N and C metabolism in cyanobacteria

S. Klähn*[1], W. R. Hess[1]
[1]*University of Freiburg, Institute for Biology III, Genetics & Experimental Bioinformatics, Freiburg, Germany*

Bacteria possess numerous and diverse means of gene regulation using non-coding, small RNAs (sRNAs). These sRNAs can activate or repress gene expression at posttranscriptional level by complementary base pairing and contribute to the specific and customized synthesis of the respective proteins. Recent RNA-seq-type transcriptomic analyses of the cyanobacterial model strain *Synechocystis* sp. PCC 6803 revealed a high number of potentially regulatory sRNAs with intriguing expression patterns. For instance, the expression of the nitrogen stress induced RNA 4 (NsiR4) is stimulated through nitrogen (N) limitation via NtcA, the global transcriptional regulator of genes involved in N metabolism. NsiR4 is widely conserved throughout the cyanobacterial phylum, suggesting a conserved function. Recently, it was verified that NsiR4 is involved in controlling nitrogen assimilation by targeting the mRNA of the *gifA* gene which encodes an inhibitory protein for the glutamine synthetase [1]. Moreover, with *ssr1528* a hitherto uncharacterized nitrogen-regulated gene was identified as a second target. However, additional *in silico* data suggest that the NsiR4 regulon is more

wide-ranging and also includes genes encoding enzymes of the Calvin-Benson-Bassam cycle, glycogen synthesis and the photosynthetic electron transport chain. All these enzymes appear to represent central hubs determining specific routes of carbon (C) metabolism. Interestingly, consistent with the N-regulated expression of NsiR4 the respective genes show altered transcript levels upon N-limitation in *Synechocystis*. However, the underlying mechanisms are unknown. Thus it is tempting to speculate that this regulation could at least partially be mediated by NsiR4. Consequently, NsiR4 might not only be involved in controlling N assimilation by fine-tuning the activity of glutamine synthetase indirectly but could also influence important C routes by targeting genes for corresponding key enzymes.

References

[1] Klähn S, Schaal C, Georg J, Baumgartner D, Knippen G, Hagemann M, Muro-Pastor AM, and Hess WR. (2015). The sRNA NsiR4 is involved in nitrogen assimilation control in cyanobacteria by targeting glutamine synthetase inactivating factor IF7. *Proceedings of the National Academy of Sciences of the United States of America*, 112 (45): E6243-E6252.

Presentation: Monday, 6 March 2017 from 19:45 - 20:00 in room 6.

SHORT LECTURE 16
Element cycles and species interactions (SL EE)
07 March 2017 • 08:30 – 10:00

095/EEV

Linking the C, N and S cycle: microbial competition and cooperation in a laboratory-scale bioreactor model

A. Arshad[1], M. Jetten[1,2], H. Op den Camp[1], C. Welte*[1,2]
[1]*Radboud University, Nijmegen, Netherlands*
[2]*Soehngen Institute of Anaerobic Microbiology, Nijmegen, Netherlands*

Introduction: In the environment, one of the main drivers in microbial community composition is the availability of substrates for growth and energy conservation. Microorganisms compete for substrates, or may be dependent on the product(s) of microbial metabolism. We are investigating a microbial community enriched from the environment that simultaneously converts ammonium, nitrate, sulfide and methane by the action of four main microbial players that reciprocally compete for substrates but also cooperate for keeping the concentration of toxic intermediates such as nitrite and sulfide low.
Objectives: We want to understand anaerobic microbial cooperation and competition for environmentally relevant substrates.
Materials and methods: Our approach is to investigate stable cultures of anaerobic chemolithoautotrophic microbial communities enriched from the environment in a small-scale bioreactor setup. The bioreactor received moderately saline mineral medium amended with ammonium, nitrate, methane and sulfide representing an anoxic brackish environment. After about 4 months, a stable microbial community had established. We investigated the metabolic interactions by monitoring the substrate conversion of the whole bioreactor and by batch assays with stable ^{13}C and ^{15}N isotopes to track specific metabolic pathways. Furthermore, the bioreactor was monitored by fluorescence in situ hybridization and by metagenome sequencing.
Results: The bioreactor showed stable conversion of all added substrates (NH_4^+, NO_3^-, CH_4, S^{2-}) over a period of more than 180 days. We found that methane was converted by nitrate-dependent *Methanoperedens*-like archaea and by the nitrite-dependent bacterium *Methylomirabilis oxyfera*. The latter was dependent on nitrite provided by *Methanoperedens*-like archaea but also competed for the substrate methane. Nitrite competition was also present as anaerobic ammonium oxidizing (anammox) bacteria were active in the bioreactor. Sulfide added to the bioreactor was completely removed by a yet-unidentified sulfide-dependent nitrate reducer/denitrifier that competed with the Methanoperedens-like archaea for nitrate but also supplied nitrite for anammox bacteria and *M. oxyfera*.
Conclusion: We were able to demonstrate that nitrite- and nitrate-dependent anaerobic methane oxidizers, anammox bacteria and sulfide-dependent denitrifiers are able to form a stable community. Furthermore, we have quantified the amounts of substrates converted by the individual microbial players. Our results contribute to understand how coastal, brackish microorganism cooperate and compete. Additionally, our community may be a model for the anaerobic treatment of moderately saline waste water.

Presentation: Tuesday, 7 March 2017 from 8:30 - 8:45 in the Franconia Hall.

096/EEV

Microbial induced corrosion – development of new analytical tools to study biofilm formation and corrosion processes

J. Sameith *[1], K. Schwibbert[1], H. Tschiche[2], N. Epperlein[3], F. Menzel[1], K. Hoffmann[2], J. Krüger[3], U. Resch-Genger[2], H. J. Kunte[1]
[1]*Bundesanstalt für Materialforschung und -Prüfung, Biologische Materialschädigung und Referenzorganismen, Berlin, Germany*
[2]*Bundesanstalt für Materialforschung und -Prüfung, Biophotonik, Berlin, Germany*
[3]*Bundesanstalt für Materialforschung und -Prüfung, Technologien mit Nanowerkstoffen, Berlin, Germany*

Microbially induced corrosion (MIC) is a severe cost and risk factor for the oil and gas industry, water heat- and cooling systems, waste water management, and infrastructural constructions. The industrial preventions management of MIC focuses on eliminating present microorganisms, which leads to the repeated and time-consuming application of biocides. However, biocides are often only minor effective for microorganisms that live protected from these agents in a biofilm matrix formed on material surfaces [1]. In recent years, microbiologist have renewed our vision on the electrochemical processes underlying material corrosion [2] induced by sulfate reducing bacteria, but the knowledge at the level of multispecies biofilm formation and the effects of material"s properties are still rather scarce.
Therefore, an interdisciplinary team of material scientists, chemists and microbiologists, established a MIC-research platform at the Federal Institute for Materials Research and Testing (BAM) to study the effects of material"s properties and biofilm formation on MIC. Using femtosecond laser pulse technology steel surfaces with defined nanostructures were manufactured. The test pieces were incubated using a flow-through chambers setup [3] with different organisms relevant for biofilm studies. Our studies showed, that nanoscale structures severely affect the biofilm thickness and, hence, growth for health threatening organisms like *E. coli* and *S. aureus*, but also for MIC-relevant bacteria such as Fe(III)-reducing *Shewanella* spec.
Another aspect of the biofilm matrix is the establishment of microscale environments with conditions, such as anaerobic or acidic microenvironments, which promotes a diverse multispecies community. However, the detection and visualization of such small-scale microenvironments is rather challenging. Further, it could directly indicate the activity of microorganism influencing or inducing corrosion processes. Therefore, dye-stained nanoscale particles, bearing pH-responsive fluorophores at the surface, were developed to measure the pH at the biofilm"s surface and within the matrix using confocal laser scanning microcopy [4]. The particles will be further advanced to measure other environmentally relevant parameters, including oxygen concentration, and concentrations of selected metal ions indicative

of corrosion of e.g., materials used for water pipelines. In the future, our platform and its analytical tools will be employed to study the formation of biofilms in dependency of the respective support material, its surface properties like roughness, and the microbial community.

References

[1] Videla H.A. and L.K. Herrera (2005) in Int Microbiol, 8: 169-80.
[2] Dinh H.T., *et al.* (2004) in Nature, 427: 829-32.
[3] Menzel F., *et al.* (2016) in Materials, 9: 770.
[4] Behnke T., *et al.* (2012) in Dyes and Pigments, 94: 247-257.

Presentation: Tuesday, 7 March 2017 from 8:45 - 9:00 in the Franconia Hall.

097/EEV

Microbial P transformation in agricultural soils

M. Grafe*[1,2], M. Görs[3], C. Baum[3], M. Schloter[1,4], S. Schulz[1]
[1]*Helmholtz Zentrum München, Research Unit Environmental Genomics, Oberschleißheim, Germany*
[2]*Technical University of Munich, Chair of Plant Nutrition, Freising, Germany*
[3]*University of Rostock, Department of Soil Science, Rostock, Germany*
[4]*Technical University of Munich, Chair of Soil Science, Freising, Germany*

Phosphorus (P) is an essential element for all life on earth. In agricultural management P fertilization is a fundamental component. Its future availability and environmental impact is disputed. Microorganisms take on a central function in the mobilization of phosphate in soil, thus making it available to plants. It is well known that fertilization can have an impact on microbial diversity and P mobilization. Especially as microbes strive to maintain their specific C:N:P stoichiometry. Therefore, we assume that amending soil with additional carbon and nitrogen alone could increase the availability of P already present in soil by enhancing microbial P mobilization.
To test this hypothesis, we took samples from the topsoil layer in spring and autumn from two long-term field trials located in Rostock and Freising, Germany. Two treatments were sampled, namely plots amended with organic fertilizer and control plots without additional fertilizer. During the time of sampling maize was planted at both sites. To reconstruct the microbial P turnover we performed direct shot gun sequencing of directly extracted DNA from the soil samples using a well-established pipeline based on Illumina® Miseq® sequencing technology. The obtained reads were quality filtered and then blasted against the NCBI non-redundant (nr) protein sequences database as well as the KEGG database using DIAMOND and assigned by means of MEGAN.
Even though both sites differed in texture, climatic conditions and soil pH, we found a surprisingly comparable core microbiome (79% overlap) when looking at genes involved in P turnover. However, *Verrucomicrobia subdivision 3* dominated in Freising, whereas *Acidobacteriaceae* dominated in Rostock. At the same time microbial P content was increased in plots that received organic fertilizers compared to control plots where no fertilizer was applied. Metagenomic data indicated that this was achieved by enhanced P uptake and stringent P starvation control. Genes for highly efficient phosphate-specific transporter (Pst) and the phosphate-inorganic transporter (Pit) as well as *phoR* and *phoB*, which are responsible for P starvation response regulation, were most abundant. Regarding P mineralization also enzymes controlled by the PhoBR two-component system were enhanced such as acid and alkaline phosphatases. This underlines the importance of efficient microbial uptake systems as well as effective gene regulation under variable P conditions. However, samples taken in Freising showed a switch in transporter preference, between spring and autumn (Pore Protein E/Pit - spring *vs*. Phn/Pst - autumn). Overall P uptake activity was dominated by *Verrucomicrobia subdivision 3*, while mineralization of P was dominated by *Sphingomonadaceae*. Taking all our data together it could be assumed that there is a possibility of differentiated steering of P transforming microbes in soil by the type of fertilizer.

Presentation: Tuesday, 7 March 2017 from 9:00 - 9:15 in the Franconia Hall.

098/EEV

The functional role of environmental spirochetes at contaminated sites

X. Dong*[1,2], C. Greening[3], T. Brüls[4], R. Conrad[5], S. Blaskowski[6], F. Kaschani[6], M. Kaiser[6], R. Meckenstock[1]
[1]*Universität Duisburg-Essen , Fakultät für Chemie - Biofilm Centre , Essen, Germany*
[2]*Helmholtz Zentrum München, Institute of Groundwater Ecology, Neuherberg, Germany*
[3]*Monash University, School of Biological Sciences, Clayton, Australia*
[4]*Institut de Génomique, Genoscope, Evry, France*
[5]*Max Planck Institute for Terrestrial Microbiology, Department of Biogeochemistry, Marburg, Germany*
[6]*Universität Duisburg-Essen, Centre of Medical Biotechnology, Essen, Germany*

Despite the frequent detection of spirochetes at contaminated sites, their role in such ecosystems remains unclear, so far. To elucidate the functional role of environmental spirochetes, we took a sulfate-reducing, naphthalene-degrading enrichment culture, which consists of a sulfate-reducing deltaproteobacterium *Desulfobacterium naphthalenivorans* and a novel spirochete which we named *Rectinema cohabitans*. We firstly constructed a metabolic profile of *R. cohabitans* by genome sequencing and shotgun proteome analysis. The data suggested that it is an obligate fermenter which catabolizes proteins and carbohydrates resulting in acetate, ethanol, and hydrogen production. Physiological experiments and genomic analyses further showed that interspecies hydrogen transfer links the two bacteria in the enrichment culture. [FeFe]-hydrogenases catalyze fermentative H_2 production by *R. cohabitans* while [NiFe]-hydrogenases couple sulfate-dependent H_2 oxidation by *D. naphthalenivorans*. Furthermore, differential proteomics and physiological experiments indicated that *R. cohabitans* utilizes dead biomass (proteins and sugars) released from *D. naphthalenivorans*. Comparative genome analysis indicated that other uncultured and isolated spirochetes are hydrogen-producing fermentative microbes similar to *R. cohabitans*. Our results indicate that fermenting spirochetes can thrive in highly toxic contaminated habitats by scavenging dead biomass concomitant with hydrogen production. The hydrogen can be consumed by other respiratory bacteria or methanogenic archaea via interspecies hydrogen transfer, thereby recycling dead biomass. Thus environmental spirochetes constitute a microbial loop in such extreme subsurface habitats.

Presentation: Tuesday, 7 March 2017 from 9:15 - 9:30 in the Franconia Hall.

099/EEV

Interkingdom Cross-Feeding of Ammonium from Marine Methylamine-Degrading Bacteria to the Diatom *Phaeodactylum tricornutum*

K. Zecher*[1], M. Suleiman[1], O. Yücel[1], N. Jagmann[1], B. Philipp[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

Methylamines occur ubiquitously in the oceans and can serve as carbon, nitrogen and energy source for heterotrophic bacteria from different phylogenetic groups within the marine bacterioplankton. In contrast, diatoms, which constitute a large part of the marine phytoplankton, are believed to be incapable of using

methylamines as nitrogen source. Photoautotrophic diatoms are known to be closely associated with heterotrophic bacteria, and it has been shown that bacteria actively approach the so-called phycosphere, in which they can utilize organic compounds released by the diatoms; in turn, diatoms may also profit from bacterial metabolites. Considering these diatom-bacteria interactions, the hypothesis was raised that methylotrophic bacteria may provide ammonium to diatoms by degradation of methylamines. This hypothesis was investigated with the diatom *Phaeodactylum tricornutum* and monomethylamine (MMA) as substrate. Bacteria supporting photoautotrophic growth of *P. tricornutum* with MMA as sole nitrogen source could be isolated from sea water. Two strains, *Donghicola* sp. strain KarMa, which harbored genes for both monomethylamine dehydrogenase and the *N*-methylglutamate-pathway, and *Methylophaga* sp. strain M1, which catalyzed MMA oxidation by MMA-dehydrogenase, were selected for further characterization. While strain M1 grew with MMA as sole substrate, strain KarMa could utilize MMA as nitrogen source only when e.g. glucose was provided as carbon source. With both strains release of ammonium was detected during MMA utilization. In co-culture with *P. tricornutum*, strain KarMa supported photoautotrophic growth with 2 mM MMA to the same extent as with the equimolar amount NH_4Cl. In co-culture with strain M1, photoautotrophic growth of *P. tricornutum* was also supported but to a much lower degree than by strain KarMa.
This proof-of-principle study[1] with a synthetic microbial community adds ammonium transfer from methylamine-degrading bacteria to the list of bacteria-diatom interactions[2]. This interkingdom cross-feeding could be an important contribution to phytoplankton growth, which has been overlooked so far.

References
[1] Suleiman, Zecher, Yücel, Jagmann & Philipp (2016). Interkingdom Cross-Feeding of Ammonium from Marine Methylamine-Degrading Bacteria to the Diatom *Phaeodactylum tricornutum*. Appl Env Microbiol 82:7113-7122.
[2] Amin, Parker, Armbrust (2012). Interactions between diatoms and bacteria. Microbiol Mol Biol Rev 76**:**667–684.

Presentation: Tuesday, 7 March 2017 from 9:30 - 9:45 in the Franconia Hall.

100/EEV
Proteomic insights into the interaction of *Marinobacter adhaerens* with diatoms
V. Will*[1], M. Ullrich[1]
[1]Jacobs University Bremen, Life Sciences and Chemistry, Bremen, Germany

The interaction between heterotrophic bacteria and diatoms play an important role in the oceanic carbon cycle. Diatoms fix CO2 and can bury the carbon in the deep ocean by sinking down due to aggregate formation. This process can be enhanced by bacteria. Previously a model system had been established to study diatom-bacteria interactions that consists of the bacterium *Marinobacter adherens* HP15 and the diatom *Thalassiosira weissflogii* (Sonnenschein et al. 2011). Our studies showed that bacteria can increase the release of transparent exopolymer particles (TEP) by diatoms that act as glue between cells and are thus important for aggregate formation (Gärdes at al. 2011).
The goal of this study was to identify proteins that are differentially expressed when bacteria directly interact with diatoms as compared to free living bacteria to get a hint which processes play a role in diatom-bacteria interactions.
M. adhaerens HP15 was co-cultivated with *T. weissflogii* in attachment assays and roller tank experiments. Subsequently, bacteria that interact with the diatom were separated from free living bacteria by filtration. In order to identify proteins that are higher expressed in free living versus diatom-attached bacteria 2D-SDS-PAGE was performed. Significantly differentially expressed proteins were identified via matrix assisted laser desorption ionization-time of flight mass spectrometry.
Microscopic observations showed that attached bacteria are mainly found inside the TEP rather than directly on the diatom cell surface. Proteomics analysis revealed that proteins higher expressed in attached bacteria play a role in the amino acid metabolism whereas free bacteria show a higher expression of motility related proteins.
These results prove our previous finding that amino acid metabolism related proteins are up-regulated during the interaction (Stahl & Ullrich 2016). We conclude that specific amino acids are located in the TEP and may act both as chemoattractant and carbon or nitrogen source for bacteria. Future experiments will include the analysis and quantification of amino acids inside the TEP compared to surrounding medium to prove this hypothesis.

References
[1] Gärdes, A., M. H. Iversen, H. P. Grossart, U. Passow and M. S. Ullrich (2011). "Diatom-Associated Bacteria Are Required for Aggregation of *Thalassiosira weissflogii*." *Isme Journal 5*(3): 436-445.
[2] Sonnenschein, E. C., A. Gärdes, S. Seebah, I. Torres-Monroy, H. P. Grossart and M. S. Ullrich (2011). "Development of a Genetic System for *Marinobacter adhaerens* HP15 Involved in Marine Aggregate Formation by Interacting with Diatom Cells." *Journal of Microbiological Methods 87*(2): 176-183.
[3] Stahl, A. & Ullrich, M. (2016). "Proteomics analysis of the response of the marine bacterium *Marinobacter adhaerens* HP15 to the diatom *Thalassiosira weissflogii*." *Aquatic Microbial Ecology* DOI: 10.3354/ame01804.

Presentation: Tuesday, 7 March 2017 from 9:45 - 10:00 in the Franconia Hall.

SHORT LECTURE 17
RNA-based Regulation and Beyond (SL GR)
07 March 2017 • 08:30 – 10:00

101/GRV
Self-control - A small RNA in bacterial photosynthesis
K. Müller*[1], G. Klug[1]
[1]Justus-Liebig-University Gießen, Institute for Micro- and Molecular biology , Gießen, Germany

Question: The photosynthetic model bacterium *Rhodobacter sphaeroides* faces photooxidative stress by the bacteriochlorophyll-mediated generation of singlet oxygen ($^1O_2$) in the light. This leads to a need for a tight regulation of the formation of photosynthetic complexes in the presence of oxygen. Our group intensively investigated this regulatory network and identified a small RNA (sRNA), namely PcrZ [1], which controls a small subset of photosynthetic genes. We therefore further focused on other sRNAs with a role in photosynthesis gene regulation.
Methods and Results: Essential for the development of the two light harvesting complexes (LHC I and LHC II) and the reaction center, are the genes encoded in the *puf* and the *puc* operons. RNAseq data from these photosynthetic gene clusters indicated the presence of an sRNA, preliminarily called PcrX, directly downstream of the last gene in the operon, *pufX* [2]. By applying Northern blot analyses and an RT-PCR set-up we found substantial evidence that PcrX is co-transcribed together with the polycistronic *puf* mRNA, even though the RNase responsible for the PcrX processing from the 3'-UTR remains elusive. Interestingly, using a *lacZ*-based *in vivo* reporter system and an

EMSA approach we could show a direct interaction between PcrX and the *pufX* part of the *puf* mRNA. An artificial increase of the sRNA by plasmid driven over-expression led to a decrease of growth under phototrophic conditions, as well as the pigmentation and LHC amount in the cells. Moreover, the PcrX over-expression phenotype is accompanied by a reduced abundance and half-life of the *puf* mRNA.
Conclusion: Taken together these data lead to the assumption that PcrX represents the second sRNA involved in the photosynthesis gene regulation of *R. sphaeroides*. Interestingly, the *puf* mRNA seems to entail its own expression control via the 3'-UTR derived sRNA PcrX.

References
[1] Mank NN, Berghoff BA, Hermanns YN, Klug G (2012) Regulation of bacterial photosynthesis genes by the small non-coding RNA PcrZ. *PNAS* 109(40):16306-11
[2] Berghoff BA, Glaeser J, Sharma CM, Vogel J, Klug G. (2009) Photooxidative stress-induced and abundant small RNAs in Rhodobacter sphaeroides. Mol Microbiol. 74(6):1497-512.

Presentation: Tuesday, 7 March 2017 from 8:30 - 8:45 in the Barbarossa Hall.

102/GRV
Inverse toeprinting: A novel method to map the primary sequence determinants of macrolide-sensing arrest peptides
B. Seip*[1,2,3], S. Arenz[4], D. Dupuy[1,2,3], D. N. Wilson[4], C. A. Innis[1,2,3]
[1]*Institut Européen de Chimie et Biologie, Pessac, France*
[2]*INSERM, U1212, Bordeaux, France*
[3]*CNRS, UMR 5320, Bordeaux, France*
[4]*Department of Chemistry and Biochemistry, Gene Center Munich, Munich, Germany*

During ribosomal protein synthesis the growing polypeptide travels through the exit tunnel spanning the large ribosomal subunit in order to reach the cytoplasm. While being translated, specific arrest peptide sequences can interact with the ribosomal tunnel wall, often with the help of a small molecule ligand, and immobilize the ribosome on the mRNA. This process, termed nascent chain-mediated translational arrest, regulates the expression of downstream genes through transcriptional or translational mechanisms. Known arrest sequences have been grouped into several classes based on their arrest motifs, but share virtually no sequence similarity with one another. A well-studied group of arrest peptides are the Erm leader peptides, which regulate the expression of the methyltransferases that confer resistance to MLS antibiotics (macrolide, lincosamide, streptogramin B).
In order to systematically identify the primary sequence determinants that lead to translational arrest, we have developed a novel high-throughput *in vitro* selection technique, which we refer to as *inverse toeprinting*. This method allows us to obtain precise and extensive information on the specific sequence requirements for drug-dependent translational arrest. We have applied inverse toeprinting to an mRNA library consisting of $>10^{11}$ *ermDL* variants and used it to drive translation *in vitro* in the presence of several macrolide antibiotics. We identified point mutations that define the selectivity of ErmDL for these drugs, in agreement with our structural data on an ErmDL-70S ribosome complex.
We further plan to extend inverse toeprinting to identify sequences that cause translational arrest in response to the small molecules of our choice. Thus, our approach provides a valuable tool to investigate the molecular principles underlying nascent chain-mediated translational arrest.

Presentation: Tuesday, 7 March 2017 from 8:45 - 9:00 in the Barbarossa Hall.

103/GRV
Tracking the elusive function of the non-coding RNA SolB from *Clostridium acetobutylicum* in solventogenesis
E. Riester*[1], T. Zimmermann[1], S. Linder[1], S. Flitsch[1], B. Schiel-Bengelsdorf[1], A. Poehlein[2], R. Daniel[2], P. Dürre[1]
[1]*Institut für Mikrobiologie und Biotechnologie, University Ulm, Ulm, Germany*
[2]*Department of Genomic and Applied Microbiology & Goettingen Genomics Laboratory, Georg-August-University Goettingen, Goettingen, Germany*

Introduction & Objectives: Small non-coding regulatory RNAs act as one of the central players for regulation in bacterial cells. Mostly, they operate as posttranscriptional regulators by binding to distinct mRNA targets. This interaction can regulate mRNA stability and translation initiation. Binding and stability of noncoding RNAs are often influenced by the RNA-binding protein Hfq.
Here, we focus on non-coding RNA SolB from the Gram-positive *Clostridium acetobutylicum.* This solvent-producing anaerobic bacterium is of importance as it naturally produces butanol, an important bulk chemical as well as biofuel. Aim of this work was to examine which part SolB plays in solventogenesis of *C. acetobutylicum.* Therefore, we elucidated the operation principle of SolB by identifying binding partners and analyzing the role of Hfq.
Methods: Northern blot, primer extension, and RT-PCR experiments were performed to provide evidence of existence of SolB. Different overexpression plasmids were constructed and transformed into *C. acetobutylicum*. Respective mutant strains and *solB* deletions strain were analyzed for growth, sporulation, copy number of the megaplasmid pSOL1 via qRT-PCR, and product formation via GC and HPLC. Furthermore, binding partners and motifs were predicted in silico. Transcriptome analysis was conducted for *C. acetobutylicum* wild type, *solB* overexpression mutant, and Δ*spo0A* mutant. Recombinant produced Hfq was tested for binding and stabilization of in vitro transcribed SolB. *hfq* was inactivated by integration and the respective strain was transformed with the *solB* overexpression plasmid to examine whether Hfq is necessary for the function of SolB.
Results: *C. acetobutylicum solB* overexpression strain blocked solvent formation and reduced sporulation and copy number of the megaplasmid pSOL1. *C. acetobutylicum solB* deletion strain did not show differences to the wild type under standard conditions. Transcripts of *spo0A* and *adhE2* were predicted as putative binding partners. Overexpression of *solB* and *spo0A* in *C. acetobutylicum* showed wild type level of solvents and pSOL1. Transcriptome data showed similarities between the influenced genes of *C. acetobutylicum solB* overexpression strain and Δ*spo0A* deletion strain. Presence of Hfq leads to a faster degradation of SolB and a distinct level of Hfq is essential for function of SolB.
Conclusion: SolB seems to be a global regulatory element for *C. acetobutylicum* as it influences central pathways such as solventogenesis and sporulation. It acts as an antagonist to the master regulator Spo0A, which plays a key role in sporulation and solvent production. As SolB displays low expression level under standard conditions and deletion did not cause phenotypic changes, SolB seems to be a tool for fine regulation and a useful instrument to prohibit early solvent production and sporulation.

Presentation: Tuesday, 7 March 2017 from 9:00 - 9:15 in the Barbarossa Hall.

104/GRV
Effect of the *rne*Δ*MTS* mutation on mRNA stability.
L. Hadjeras*[1], L. Poljak[1], L. Girbal[2], Q. Morin-Ogier[1], I. Canal[1], M. Cocaign-Bousquet[2], M. Bouvier[1], A. J. Carpousis[1]
[1]*CNRS & Université Paul Sabatier, Laboratoire de Microbiologie et Génétique Moléculaires, Toulouse, France*
[2]*INRA, CNRS & Université de Toulouse, Laboratoire d'Ingénierie des systèmes biologiques et des procédés, Toulouse, France*

Although it is now well established that RNase E is localized to the inner cytoplasmic membrane of *Escherichia coli*, we do not understand the consequences of this localization for RNA maturation, surveillance and degradation. One approach to address this question is to study mutant strains in which RNase E localizes to the cytoplasm. Deletion of the *rne* Membrane Targeting Sequence (*rne*Δ*MTS*) results in a viable strain that express cytoplasmic RNase E (cRNase E). Here we show that there is a small but measurable slowdown of mRNA degradation in the *rne*Δ*MTS* strain. *In vitro* assays show that the endoribonuclease activity of cRNase E is comparable to the wild type control. Furthermore, immunoprecipitation shows that cRNase E associates with RhlB, enolase and PNPase to form a cytoplasmic RNA degradosome. These results suggest that the slowdown in mRNA degradation is not due to a defect in RNase E activity or the failure to assemble an RNA degradosome. Nevertheless, the *rne* message is stabilized in the *rne*Δ*MTS* strain and *rne-lacZ* fusions show that expression increases about 2-fold. Since *rne* expression is autoregulated, this result suggests either increased demand for RNase E activity or a defect in autoregulation. The T7-*lacZ* gene is a synthetic construct for measuring mRNA surveillance. The rapid elongation rate of T7 RNA polymerase results in the synthesis of long stretches of ribosome-free mRNA that make the message exceptionally sensitive to inactivation by RNase E. Surprisingly, the inactivation of the T7-*lacZ* mRNA is faster in the *rne*Δ*MTS* strain. This result suggests that increased levels of cRNase E accelerates the degradation of ribosome-free mRNA. Our results suggest that localization of the RNA degradosome to the inner cytoplasmic membrane is necessary for normal mRNA degradation, for control of *rne* expression and for surveillance of untranslated mRNA.

Presentation: Tuesday, 7 March 2017 from 9:15 - 9:30 in the Barbarossa Hall.

105/GRV
The Nucleoid Associated Protein HU Insulates Transcription Units in *Escherichia coli*
M. Berger*[1], U. Dobrindt[1]
[1]*UKM, Institut für Hygiene, Münster, Germany*

Introduction: Except for gene dosage effects resulting from a relative decrease in copy number from the Origin (OriC) to the Terminus of replication in growing cells, transcription units are known to be expressed largely independent of their genomic context in *Salmonella typhimurium* and *Escherichia coli (E. coli)* wild type cells. However, we observed earlier that genes upregulated in *E. coli* cells lacking the conserved nucleoid associated protein HU clustered in a chromosomal domain comprising OriC and the ribosomal RNA operons without showing a relative increase in gene copy number. In addition, the mutants were defective in the formation of so called transcription foci, spatially confined aggregations of RNA polymerases which are characteristic for rapidly growing *E. coli* K-12 wild type cells. HU is known to constrain negative supercoils which are both required for active transcription of ribosomal RNA operons, as well as generated by the transcription process itself. Therefore, these observations suggested that the expression of genes was chromosomal position-dependent in the mutant because the transcription units may have become mechanically coupled in the absence of the protein. We tested this hypothesis in more detail by designing a genetic test system for chromosomal position effects on transcription.
Results and discussion: We constructed a set of reporter strains containing different sets of promoter-fluorescent reporter gene fusion modules inserted in the same distance from OriC on both replichores of the *E. coli* K-12 chromosome that allowed the measurement of the production of the reporter gene in real time. In contrast to wild type cells the reporter modules showed various drastic chromosomal positional effects in cells lacking HU. In addition we provide strong evidence for the hypothesis that the expression of the modules is indeed directly affected by the activity of transcription units in close spatial proximity. Our results therefore suggest that the previously observed chromosomal context independence of bacterial transcription units is not as so far believed a *cis* encoded property, but conferred by a factor that is encoded in *trans*, namely the namely the conserved nucleoid associated protein HU.

Presentation: Tuesday, 7 March 2017 from 9:30 - 9:45 in the Barbarossa Hall.

106/GRV
Two small RNAs conserved in *Enterobacteriaceae* provide intrinsic resistance to antibiotics targeting the cell wall biosynthesis enzyme Glucosamine-6-phosphate synthase
M. A. Khan*[1], Y. Göpel[1], S. Milewski[1], B. Görke[1]
[1]*University of Vienna, Max F. Perutz Laboratories, Vienna, Austria*

Glucosamine-6-phosphate Synthase GlmS catalyses the initial reaction in the pathway dedicated to cell envelope biosynthesis: formation of glucosamine-6-phosphate (GlcN6P). Owing to its essentiality, GlmS has been considered as a target for antimicrobial drugs. Nva-FMDP, a synthetic di-peptide, is a potent inhibitor of GlmS. It irreversibly inhibits GlmS enzymatic activity, leading to cessation of cell wall biosynthesis and consequently cell death. Interestingly, it was observed that Gram-negative bacteria are less susceptible to the drug for so far unknown reasons. In these bacteria, GlmS is feedback regulated by a complex regulatory cascade involving two homologous small RNAs. When amounts of GlcN6P suffice, the base-pairing sRNA GlmZ is targeted to RNase E mediated decay by adaptor protein RapZ. In contrast, GlcN6P depletion triggers accumulation of sRNA GlmY, which acts as a molecular decoy and titrates the RapZ protein, thereby stabilising GlmZ. Full length GlmZ base-pairs with the *glmS* mRNA in an Hfq dependent manner, and activates its translation (1). Here we show that exposure of wild type *E. coli* cells to Nva-FMDP leads to a strong accumulation of sRNAs GlmY and GlmZ, and consequently *glmS*, allowing the cells to overcome antimicrobial action of the drug. Consequently, *glmY* or *glmZ* mutants are highly susceptible to the compound, emphasising the role of this cascade in providing intrinsic resistance to the drug. Hence, prevention of activation of the GlmY/Z cascade could increase potency of Nva-FMDP towards wild type cells. Indeed, initial experiments indicate that coadministration of a non-metabolisable GlcN6P analogue amplifies activity of Nva-FMDP against wild type bacteria through suppression of the GlmY/Z cascade, highlighting its importance as a potential target for antimicrobial chemotherapy (2). Furthermore, identification of the GlcN6P sensor would aid in optimally designing a more suitable GlcN6P analogue. Initial insight into the mechanism of GlcN6P perception by the GlmY/GlmZ system will be presented.

References
[1] Göpel, Y, Khan, MA, Görke B (2014) *RNA Biol*. 1(5):433-42
[2] Khan, MA, Göpel, Y., Milewski, S., Görke, B. (2016) *Front. Microbiol*. 44(2):824-37

Presentation: Tuesday, 7 March 2017 from 9:45 - 10:00 in the Barbarossa Hall.

MINISYMPOSIA 18
Multiple Omics for Exploring Microbial Physiology and Pathogenicity (FG FG)
07 March 2017 • 08:30 – 10:00

107/FGV

Metatranscriptomic analysis of bovine rumen microbiome dynamics during plant biomass degradation

T. Urich*[1]
[1]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*

Ruminant livestock is the major anthropogenic source of the potent greenhouse gas methane. A complex rumen microbiota consisting of Bacteria, Eukarya and methanogenic Archaea accomplishes the anaerobic degradation of plant biomass in ruminants. Understanding their activities and tightly coupled interactions during feed degradation is essential to eventually develop sustainable methane mitigation strategies.
Using an integrated approach, combining quantitative metatranscriptomics with gas and short chain fatty acid (SCFA) profiling, we investigated the temporal rumen microbiome dynamics during plant biomass degradation in lactating cows.
The rumen microbiome (including pro- and eukaryotic viruses) was highly individual and remarkably stable within each cow rumen fluid (RF), suggesting a high functional redundancy among taxa for plant biomass degradation. Nevertheless, a consistent successional pattern could be observed, with bacterial (e.g. *Succinivibrionaceae*) and eukaryotic taxa (e.g. *Mastigamoeba*) significantly more abundant in earlier and later time-points, respectively. Gene expression profiles revealed a fast growth response of the microbiota, reflected by a drastic increase of rumen microbial biomass, methane emissions and SCFA concentration. Accordingly, transcription of various glycoside hydrolases was induced upon feed intake and returned to low before-feeding abundances five hours after feeding. Functional transcripts of the dominant methanogen taxa *Methanobrevibacter, Methanomassiliicoccales*[AS1] *and Methanosphaera* showed a similar, albeit less pronounced trend[AS2] .
This first comprehensive longitudinal study of the complex rumen microbiome during feed degradation revealed a defined sequence of events and interactions between microbiota members that might be exploited to mitigate methane emissions from ruminants in the future.

Presentation: Tuesday, 7 March 2017 from 8:30 - 8:45 in room 13.

108/FGV

Application of meta-omics approaches in microbial ecology

B. Wemheuer*[1]
[1]*Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Göttingen, Germany*

Bacteria are essential for ecosystem stability and functioning. Assessing the response and/or resilience of these microbes towards environmental changes has thus become one fundamental issue in microbial ecology. Combining meta-omics approaches with next-generation sequencing (NGS) technologies has provided unprecedented insights into diversity and composition of bacterial communities in various habitats.
Here, we investigated the composition of bacterial communities in terrestrial as well as in marine habitats and their response towards environmental changes using metabarcoding targeting 16S rRNA genes and transcripts. To study microbial functioning, we predicted functional profiles (artificial metagenomes and metatranscriptomes) from obtained 16S rRNA data using the novel bioinformatic tool Tax4Fun.
We studied bacterial endophyte communities in aerial plant parts of three agricultural important grass species and evaluated the impact of fertilizer application and mowing frequencies on these communities. Obtained results revealed that grass species rather than management regimes drive bacterial endophyte composition and function. Moreover, compositional and functional community patterns showed no correlation to each other indicating that plant species-specific selection of endophytes is driven by functional rather than phylogenetic traits. In another study, we investigated structural and functional responses of rhizosphere bacterial communities towards fertilizer application, mowing frequencies and above-ground herbivory under herbicide application against monocots and dicots, respectively. Herbicide application significantly affected bacterial communities and their response towards applied management regimes. In addition to terrestrial habitats, we studied bacterioplankton communities in the North Sea. Here, we used functional predications in combination with structural modelling approaches to decipher ecological traits of abundant marine bacterial lineages.
The combination of meta-omics approaches and NGS technologies with novel bioinformatic tools has provided valuable insights into compositional and functional responses of microbial communities in various habitats towards environmental changes.

Presentation: Tuesday, 7 March 2017 from 8:45 - 9:00 in room 13.

109/FGV

Protein *S*-bacillithiolation functions in thiol-protection and redox regulation of the glyceraldehyde-3-phosphate dehydrogenase Gap in *Staphylococcus aureus* under hypochlorite stress

M. Imber[1], N. T. T. Huyen[1], A. J. Pietrzyk-Brzezinska[2], V. V. Loi[1], M. Hillion[1], J. Bernhardt[3], L. Thärichen[4], K. Kolsek[4], L. Adrian[5], F. Gräter[4], M. Wahl[2], H. Antelmann*[1]
[1]*Freie Universität Berlin, Institute for Biology-Microbiology, Berlin, Germany*
[2]*Freie Universität Berlin, Laboratory for Structural Biochemistry, Berlin, Germany*
[3]*Ernst-Moritz-Arndt-University of Greifswald, Institute for Microbiology, Greifswald, Germany*
[4]*Heidelberg University, Molecular Biomechanics, Interdisciplinary Center for Scientific Computing (IWR), Heidelberg, Germany*
[5]*Helmholtz Centre for Environmental Research-UFZ, Department Isotope Biogeochemistry, Leipzig, Germany*

Introduction*:* Bacillithiol (BSH) is the major low molecular weight (LMW) thiol and maintains the thiol-redox balance of the human pathogen *Staphylococcus aureus*. In previous studies, we have shown that BSH functions in post-translational thiol-modification by protein *S*-bacillithiolation under hypochlorite stress in related Firmicutes.
Objectives: In this study, we were interested to identify novel redox-sensitive thiol-switches and *S*-bacillithiolated proteins under infection-related conditions in *S. aureus*.
Materials & Methods: We used the mass spectrometry-based redox proteomics approach OxICAT and *Voronoi redox treemaps* to quantify hypochlorite-sensitive protein thiols in *S. aureus* USA300 and analyzed the role of BSH in protein *S*-bacillithiolation by shotgun proteomics.
Results: The OxICAT analyses enabled the quantification of 228 Cys residues in the redox proteome of *S. aureus* USA300. Hypochlorite stress resulted in a >10% increased oxidation of 58 Cys residues (25.4 %) in the thiol-redox proteome. Among the highly oxidized NaOCl-sensitive proteins are five *S*-bacillithiolated proteins (Gap, AldA, GuaB, RpmJ and PpaC). The glyceraldehyde-3-phosphate dehydrogenase Gap represents the

most abundant *S*-bacillithiolated protein contributing with 4% to the total Cys proteome. The active site Cys151 of Gap was very sensitive to overoxidation and irreversible inactivation by H2O2 or NaOCl *in vitro*. Treatment with H2O2 or NaOCl in the presence of BSH resulted in reversible Gap inactivation due to *S*-bacillithiolation, which could be regenerated by the bacilliredoxin Brx (SAUSA300_1321) *in vitro*. Molecular docking was used to model the *S*-bacillithiolated Gap active site suggesting that formation of the BSH mixed disulfide does not require major structural changes. **Conclusion:** Using OxICAT analyses, we identified 58 novel NaOCl-sensitive proteins in the pathogen *S. aureus* that could play protective roles against the host immune defense and include the glycolytic Gap as major target for *S*-bacillithiolation. *S*-bacillithiolation of Gap did not require structural changes, but efficiently functions in redox regulation and protection of the active site against irreversible overoxidation in *S. aureus*.

Presentation: Tuesday, 7 March 2017 from 9:00 - 9:15 in room 13.

110/FGV

Tracking gene expression and oxidative damage of O_2-stressed *Clostridium difficile* by a multi-omics approach

M. Neumann-Schaal[1], A. M. Nuss[2], J. Hofmann[1], A. Otto[3], M. Beckstette[2], P. Dersch[2], D. Becher[3], D. Schomburg[1], K. Riedel[4], S. Sievers*[4]
[1]*TU Braunschweig, Bioinformatics and Biochemistry & BRICS, Braunschweig, Germany*
[2]*Helmholtz Centre Infection Research, Molecular Infection Biology, Braunschweig, Germany*
[3]*University of Greifswald, Institute of Microbiology, Microbial Proteomics, Greifswald, Germany*
[4]*University of Greifswald, Institute of Microbiology, Microbial Physiology & Molecular Biology, Greifswald, Germany*

Question: The intestinal human pathogen *C. difficile* meanwhile represents the most frequent cause of hospital acquired diarrhea. Infections involve serious and recurrent inflammation of the intestinal epithelium often with a lethal outcome. As *C. difficile* represents a strict anaerobic bacterium, already low concentrations of oxygen in the intestinal tract should impair its growth. However, *C. difficile* proved to be surprisingly tolerant towards the presence of oxygen [1]. To understand this tolerance, we stressed *C. difficile* with micro-aerobic conditions and monitored its response by different omics approaches.
Methods: Exponentially growing *C. difficile* 630Δ*erm* was stressed by a gas flow of O_2/N_2 (5:95). A control culture was streamed solely with N_2. rRNA depleted total RNA from samples taken 15 min and 1 h after stress onset was converted into cDNA and sequenced. Comprehensive metabolome analyses were performed for the same time points. Additionally, oxidative damage of protein cysteines was evaluated employing a differential labelling protocol with subsequent mass spectrometric analyses of peptides.
Results: *C. difficile* shifted to micro-aerobiosis kept growing to the same extent as anaerobically growing cells for the duration tested. The more surprising it is, that we determined an extensive change in gene expression by RNA-Seq with more than 250 affected genes after 15 min and even 600 genes changing their expression by a factor of 2 or higher after 1 h. Affected genes exhibit a wide range of functionality, including numerous ABC transporters and enzymes of cell wall synthesis that are strongly induced whereas transcripts of proteins involved in purine biosynthesis and flagella formation are less abundant in presence of 5% O_2. Metabolomics affirmed the far-reaching impact of micro-aerobiosis with many metabolites changing in concentration. Furthermore, redox proteomics revealed a dramatic increase in the oxidative state of cysteine in more than 800 peptides after micro-aerobic shock, and provides a catalogue of oxidation-prone cysteine residues in the *C. difficile* proteome.
Conclusion: Abrogating *C. difficile* adaptation to oxygen and reactive oxygen species could be a starting point for the development of novel treatment strategies. However, knowledge on the oxidative stress response of the pathogen is scarce [2]. Our study suggests that *C. difficile´*s tolerance towards O_2 cannot be explained by the action of a few detoxifying enzymes, but rather by a complex and far-reaching adjustment of global gene expression.

References

[1] Edwards *et al*., 2016, Frontiers in Microbiology 7, 1698
[2] Emerson *et al*. 2008, Journal of Medical Microbiology 57, 757–64

Presentation: Tuesday, 7 March 2017 from 9:15 - 9:30 in room 13.

111/FGV

Elucidating the function of a novel drug target and an essential GTPase Obg by proteome and metabolome perturbation analysis

A. Sikora*[1], J. Bonventre[1], R. Zielke[1], I. Wierzbicki[1], J. Morré[1], J. F. Stevens[1], P. Gafken[2]
[1]*Oregon State University, College of Pharmacy , Corvallis, Oregon, United States*
[2]*Fred Hutchinson Cancer Research Center, Seattle, Washington, United States*

Neisseria gonorrhoeae (GC) is the etiologic agent of the sexually transmitted infection gonorrhea, and is rapidly acquiring antibiotic resistance. The essential bacterial GTPase Obg was proposed as a novel target for alternative antimicrobial interventions due to its association with critical cellular processes. To better understand the global physiological consequences of pharmaceutically targeting Obg, we compared the proteomic and metabolomics profiles of *Neisseria gonorrhoeae* under Obg-depleted [(-)Obg_{GC}] and wild type (wt) conditions. Quantitative proteomics identified 1038 proteins across three biological replicates, of which 159 were differentially expressed by ≥1.5 fold between wt and (-)Obg_{GC}. The majority of these proteins were upregulated (133) and located in the cytoplasm (84). Untargeted metabolomics yielded 1275 water-soluble metabolites that were significantly ($p \leq 0.05$, ≥1.2 fold) different between wt and (-)Obg_{GC}, with 48 metabolites in 61 distinct KEGG pathways. Integrating the proteomic and metabolomics datasets identified 34 common KEGG pathways, and highlighted a number of metabolism and biosynthesis pathways altered significantly by the depletion of Obg. In particular, glutathione metabolism was perturbed, supporting the role for Obg in stress response. Peptidoglycan biosynthesis, a newly discovered function associated with Obg, was also altered in both datasets.
Our results lend support to Obg's hypothesized role in bacterial persistence via the suppression of major cellular processes and provide new insights into Obg's targets and effectors, as well as a better understanding of a clinically relevant human pathogen.

Presentation: Tuesday, 7 March 2017 from 9:30 - 9:45 in room 13.

112/FGV

Diversity driven cultivation and genome sequencing of 74 slow growing Planctomycetes reveal their potential for small molecule production

S. Wiegand*[1], M. Jogler[1], A. Heuer[1], P. Rast[1], A. K. Kaster[1], O. M. Lage[2], L. Øvreås[3], L. van Niftrik[4], J. Vollmers[1], C. Jogler[4]
[1]*Leibniz Institute DSMZ, Microbial Cell Biology and Genetics, Braunschweig, Germany*
[2]*University of Porto, Porto, Portugal*
[3]*University of Bergen, Bergen, Norway*
[4]*Radboud University, Microbiology, Institute for Water & Wetland Research, Nijmegen, Netherlands*

Members of the taxon Planctomycetes, that belong to the PVC (Planctomycetes-Verrucomicrobia-Chlamydiae) superphylum, are characterized by many unusual features. They possess a compartmentalized cell architecture and divide by FtsZ-independent binary fission or polar budding. Associated with this atypical trait is their complex life cycle of sessile mother and planktonic daughter cells. Also, planctomycetal genomes encode numerous giant genes and bear the genomic potential for secondary metabolite production.
As the whole clade is notably under-sampled with relatively few genomic sequences being publicly available, we focused our efforts on increasing the data basis for comparative studies. For this purpose, we sampled multiple aquatic habitats around the globe and isolated 74 new planctomycetal strains from various biotic and abiotic surfaces. Planctomycetes were enriched by targeting the slow growing antibiotic resistant bacteria in combination with selective carbon sources. Most of these strains represent novel taxa up to the level of a new order, capturing an unpreceded degree of diversity. In total, we brought more novel Planctomycetes into pure culture than currently described species of this phylum exist.
To allow detailed analyses we produced closed as well as high-quality draft genomes of the novel isolates. The exploration of the gained genomic information not only enables deep insights into the taxonomy of the phylum and its subtaxa, but also the definition of its core and pan genome. The latter increases our knowledge on the metabolic versatility and the environmental role of this phylum. Furthermore, we identified secondary metabolite related genes and further strengthened our hypothesis of Planctomycetes as talented producers. Finally, the dataset will lead to the development of an advanced hypothesis of planctomycetal cell division.

Presentation: Tuesday, 7 March 2017 from 9:45 - 10:00 in room 13.

113/FGV

Chronic infection by *p. Aeruginosa* in cystic fibrosis: induction of dysbiosis through pyocyanin

S. Boutin*[1,2], L. Hennemann[1], M. Stahl[3,4,2], S. Y. Graeber[3,4,2], S. Dittrich[4,5,2], M. Mall[3,4,2], A. Dalpke[1,2]
[1]*University of Heidelberg, Department of infectious disease, Medical Microbiology and Hygiene, Heidelberg, Germany*
[2]*Translational Lung Research Center Heidelberg, Member of the German Center for Lung Research (DZL), Heidelberg, Germany*
[3]*University of Heidelberg, Div. of Pediatric Pulmonology & Allergology and Cystic Fibrosis Center, Dept. of Pediatrics,, Heidelberg, Germany*
[4]*University of Heidelberg, Dept. of Translational Pulmonology, Heidelberg, Germany*
[5]*University Hospital Heidelberg, Dept. of Pneumology and Critical Care Medicine, Thoraxklinik, Heidelberg, Germany*

Introduction: In patients with cystic fibrosis (CF), chronic infection with *Pseudomonas aeruginosa* is a major risk factor for deterioration of CF lung disease. The aim of the present study was to analyze the impact of infection by *P. aeruginosa* on the commensal airway microbiota and to study the interaction of *P. aeruginosa* with strains belonging to two commensals genera, *Streptococcus* and *Neisseria*.
Methods/Results: Using 16S amplicon sequencing, we analyzed 396 sputum samples of more than 76 CF patients to evaluate the effect of the infection on the microbiota. Patient"s infection status was classified as negative, intermittent or chronic for *P. aeruginosa* according to established criteria. We observed that patients with intermittent infection (transient detection of *P. aeruginosa* over time) still display a non infected-like microbiota with a low abundance of *P. aeruginosa*. In contrast, chronically infected patients exhibit a complete dysbiosis with dominance of *P. aeruginosa* and a global decrease of commensals. Those results indicate that *P. aeruginosa* induces dysbiosis of the microbiota via an active mechanisms and that dysbiosis only follows establishment of *P. aeruginosa* infection.
We therefore tetsed the effect of supernatants of 70 strains of *P. aeruginosa* isolates on the growth of commensals and vice versa. We also quantified biofilm formation of the various strains of *P. aeruginosa*. Our in vitro interaction assays showed that only strains producing pyocyanin strongly inhibited the growth of *Neisseria* and *Streptococcus*. We validated those results by cultivating commensals in different concentrations of pyocyanin alone to show that pyocyanin was inducing the same reduction as *P. aeruginosa* full supernatant. The proportion of pyocyanin and biofilms producers was higher in the population of patients suffering of intermittent infection than the chronically infected one indicating that the ability to produce pyocyanin and form biofilm is adaptive for the early step of infection but not selected once the infection is established.
Conclusions: Our data show that only chronic infection with *P. aeruginosa* is associated with profound changes of the lower airways" microbiota indicating that early eradication of *Pseudomonas* may prevent severe dysbiosis in patients with CF. We demonstrate that pyocyanin production by *P. aeruginosa* helps to establish dysbiosis. We also demonstrate that long term adapted strain of *P. aeruginosa* do not inhibit commensals growth and form less biofilm.

Presentation: Tuesday, 7 March 2017 from 10:00 - 10:15 in room 13.

114/FGV

Comparative Genome Analysis To Improve Detection And Typing Of Shiga toxin-expressing Escherichia coli

M. Kiel*[1], A. Leimbach[1], P. Sagory-Zalkind[2], D. Harmsen[3], A. Mellmann[1], C. Sekse[4], F. Rechenmann[2], U. Dobrindt[1]
[1]*University of Münster, Institute of Hygiene, Münster, Germany*
[2]*Genostar, Grenoble, France*
[3]*University Hospital of Münster, Department of Periodontology, Münster, Germany*
[4]*National Veterinary Institute, Oslo, Norway*

Escherichia coli (E. coli) is usually a mutual member of the human microbiota. Nevertheless, several pathogenic E. coli pathotypes exist. Especially foodborne infections with Shiga toxin-expressing E. coli (STEC), which can lead to bloody diarrhoea and the severe haemolytic uremic syndrome (HUS), are a worldwide health concern. To ensure the best treatment of patients and to facilitate strain typing and risk assessment a quick and reliable DNA-sequence-based detection method for STEC is important.
To ensure an unbiased search for possible STEC biomarkers, genome sequences of E. coli were collected from the NCBI Sequence Read Archive. The sequences were used for a molecular epidemiological analysis including Multi Locus Sequence Typing (MLST) and virulence gene profiling. Based on these results and metadata analysis, 248 sequences were grouped into STEC, other diarrheagenic E. coli, extraintestinal pathogenic E. coli (ExPEC) and commensal genomes. These groups were screened for

putative biomarkers using a bioinformatics pipeline. Our results indicate a high genomic diversity among STEC strains. As a consequence, a general biomarker set specific for all used STEC variants could not be identified. Nevertheless, further subdividing of the genomes according to clonal lineage or serotype led to the discovery of 54 putative group-specific biomarkers.
Following the in silico identification of these biomarkers, a multiplex PCR was established which reliably detects the Big Five and additional clinically important STEC subgroups. Thus, our improved DNA-based typing method can facilitate STEC typing and risk assessment in routine surveillance as well as in the food industry.

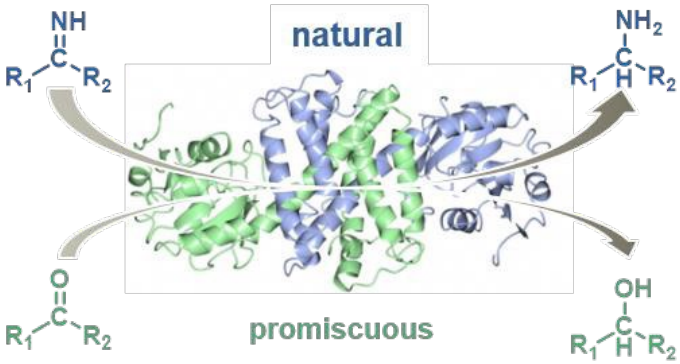
Presentation: Tuesday, 7 March 2017 from 10:15 - 10:30 in room 13.

MINISYMPOSIA 19
New enzymes and how to find them 2 (FG BT)
07 March 2017 • 08:30 – 10:00

115/BTV
Imine reductases as versatile biocatalysts

B. Nestl*[1],M. Lenz[1], N. Borlinghaus[1], L. Weinmann[1]
[1]Universitaet Stuttgart, Institute of Technical Biochemistry, Stuttgart, Germany

Chiral amines have proven to be powerful building blocks for defining new pharmaceutical and agrochemicals due to their high density of structural information. Many methods are available for chiral amine synthesis, yet surprinsingly few chiral amine structural categories can be efficiently synthesized with respect to high overall yield and enantiomeric purity. In this light, the reduction of prochiral C=N double bonds is a well-established route in synthetic chemistry due to the easy accessibility of imines from their ketone precursors with the asymmetric addition of hydrogen or a hydride as the key stereo-differentiating step. Recently, we have witnessed remarkable advances in the enzyme-catalyzed asymmetric reduction of imines by NADPH-dependent imine reductases (IREDs).[1,2] Imine reductases were presented that catalyze the asymmetric reduction of various imines and the chemo- and stereoselective reductive amination as a useful method for the preparation of amines derived from aldehydes and ketones.[3,4]
Figure 1: Asymmetric reduction of C=N and C=O double bonds by using an imine reductase.



We have further extended these successes by combining imine reductase with an engineered flavoprotein oxidase for the synthesis of saturated, substituted *N*-heterocycles. Recent work on the promiscuous behavior of imine reductases in respect of the asymmetric reductive of activated C=O double bonds will be presented.[5] Assisted by *in silico* calculations of energy barriers for the hydride transfer from the nicotinamide subunit of NAD(P)H to several imines and their corresponding iminium ions, the present results contribute to a deeper understanding of the reaction mechanism of imine reductases and their evolution.

References
[1]G. Grogan, N. J. Turner, *Chem. - A Eur. J.* **2016**, *22*, 1900–1907.
[2]J. H. Schrittwieser, S. Velikogne, W. Kroutil, *Adv. Synth. Catal.* 2015, *357*, 1655–1685.
[3]P. N. Scheller, M. Lenz, S. C. Hammer, B. Hauer, B. M. Nestl, *ChemCatChem* 2015, *7*, 3239–3242.
[4]P. N. Scheller, S. Fademrecht, S. Hofelzer, J. Pleiss, F. Leipold, N. J. Turner, B. M. Nestl, B. Hauer, *ChemBioChem* 2014, *15*, 2201–2204.
[5]M. Lenz, J. Meisner, L. Quertinmont, S. Lutz, J. Kästner, B. Nestl, *ChemBioChem* 2017, DOI 10.1002/cbic.201600647.

Presentation: Tuesday, 7 March 2017 from 8:30 - 9:00 in room 10-11.

116/BTV
Selective epoxide ring opening catalyzed by novel halohydrin dehalogenases

E. Calderini*[1], M. Schallmey[1], J. Koopmeiners[1], R. Wardenga[2], A. Schallmey[1]
[1]Technische Universität Braunschweig, Institute for Biochemistry, Biotechnology and Bioinformatics, Braunschweig, Germany
[2]Enzymicals AG, Greifswald, Germany

Halohydrin dehalogenases (HHDHs) are rare but industrially relevant enzymes as they catalyze the reversible dehalogenation of vicinal haloalcohols with formation of the corresponding epoxides.[1] In the reverse reaction, formation of new C-C, C-N, C-O or C-S bonds is possible due to their promiscuous epoxide ring opening activity using various negatively charged nucleophiles. The ring opening of terminal epoxides by HHDHs has previously been investigated, showing that the nucleophilic attack always happens on the sterically less hindered carbon atom of the epoxide ring.[2]
Recently, we identified 37 novel HHDH sequences using a motif-based sequence database mining approach.[3] Of these, 17 representative members from all phylogenetic subtypes have been biochemically and biocatalytically characterized regarding their substrate scopes, thermostability and enantioselectivity.[4]
Now, these HHDHs have also been tested for the ring opening of di-substituted epoxides using azide as nucleophile. Interestingly, the enzymes catalyze the ring opening of the tested di-substituted epoxides in a regio- and stereoselective manner exhibiting different efficiencies and selectivities.

Presentation: Tuesday, 7 March 2017 from 9:00 - 9:30 in room 10-11

117/BTV
Regio- and stereoselective intermolecular oxidative phenol coupling of microbial P450 enzymes

M. Wilde*[1], L. S. Mazzaferro[1], A. Präg[2], K. G. Hugentobler[3], M. Müller[1]
[1]Institute of Pharmaceutical Sciences, Freiburg, Germany
[2]Bachem AG, Bubendorf, Switzerland
[3]TU Braunschweig, Institute of Biochemistry, Biotechnology and Bioinformatics, Braunschweig, Germany

Cytochrome P450 enzymes (P450s) catalyze a broad variety of reactions, aside from hydroxylation reactions. The P450-catalyzed C–C bond-formation in oxidative phenol coupling often exhibits high regio- and/or stereoselectivity.
In this work, the biosynthetic dimerization steps towards biaryl polyketides were investigated in bacterial and fungal strains. The PKS gene clusters for the biosynthesis of julichromes in

Streptomyces afghaniensis, setomimycin in *S. aurantiacus,* spectomycin in *S. spectabilis,* and dimeric coumarins in *Aspergillus* spp. and *Emericella desertorum* were identified by genome analysis (Präg et al., 2014; Mazzaferro et al., 2015). Disruption of the PKS genes (*julA-C*) in *S. afghaniensis* led to complete loss of julichrome production. The gene *julI* encoding a cytochrome P450 enzyme was heterologous expressed in *E. coli.* The conversion of the monomer julichrome Q6 to the dimer julichrome Q6-6 verified that the P450 enzyme JulI is actually accountable for this coupling reaction. Likewise, the P450 enzymes KtnC from *A. niger* and DesC from *E. desertorum* were shown to catalyze the conversion of the monomer 7-demethylsiderin to the dimers orlandin and desertorin A, respectively, when expressed in *S. cerevisiae*.
In consideration of a substrate reliance of the coupling reaction, variations of the natural substrate(s) were investigated and a core structure was deduced to generate a synthetic substrate. These experiments together with the on-going crystallization of JulI (in cooperation with the group of O. Einsle, Department of Biochemistry, Albert-Ludwigs-University, Freiburg) will bring insights into the substrate specificity and reaction selectivity of the P450-catalyzed phenol coupling.

References
[1] S. Mazzaferro, W. Hüttel, A. Fries, M. Müller, *J. Am. Chem. Soc.* 2015, *137*, 12289–12295.
[2] Präg, B. A. Grüning, M. Häckh, S. Lüdeke, M. Wilde, A. Luzhetskyy, M. Richter, M. Luzhetska, S. Günther, M. Müller, *J. Am. Chem. Soc.* 2014, *136*, 6195–6198.

Presentation: Tuesday, 7 March 2017 from 9:30 - 10:00 in room 10-11

WORKSHOP 20
Bacterial metabolism and virulence (FG MP)
07 March 2017 • 08:30 – 10:00

118/MPV
Fine-tuned hydrophobicity and cognate chaperone-binding facilitate membrane protein secretion through bacterial type III secretion systems
L. Krampen*[1], S. Malmsheimer[1], I. Grin[1], J. W. de Gier[2], S. Wagner[1,3]
[1]*Interfaculty Institute of Microbiology and Infection Medicine, University Tübingen, Section of Cellular and Molecular Microbiology, Tübingen, Germany*
[2]*Stockholm University, Biochemistry and Biophysics, Stockholm, Sweden*
[3]*German Center for Infection Research (DZIF), Partner-side Tübingen, Tübingen, Germany*

Bacterial type III secretion systems inject effector proteins into eukaryotic host cells in order to promote survival and colonization of many Gram-negative pathogens and symbionts. The core unit of these systems is a cell envelope-spanning macromolecular machine termed injectisome. Most type III-secreted substrates are soluble proteins but a number of transmembrane domain containing substrates are known, of which some are even essential for the function of these systems. Transmembrane segments within type III secretion system substrates pose a targeting conflict as two sequential secretion signals for two different incompatible pathways are concatenated in the same protein: The N-terminal type III secretion signal guides export through the injectisome while sufficiently hydrophobic transmembrane segments are targeted to the bacterial inner membrane by the signal recognition particle. However, mistargeting of type III secretion system substrates to the inner membrane needs to be strictly avoided since mistargeted proteins are not only wasted but might also be toxic for the bacterium.
Here we investigated how discrimination between these two membrane protein targeting pathways is achieved inside bacteria. We show that a fine-tuned hydrophobicity of the transmembrane segments of type III secretion system substrates is one factor for targeting discrimination: While being sufficiently hydrophobic for principle membrane partitioning, signal recognition particle dependent membrane targeting is in general not supported by these segments. This observation was not only made for transmembrane domain-containing substrates of type III secretion systems but also for those of type IV secretion systems of Legionella pneumophila and Coxiella burnetti and thus seems to be a more general mechanism of targeting discrimination. A second factor for targeting discrimination, at least for some type III secretion system substrates, is the binding of their transmembrane segments by their cognate chaperones. This chaperone binding even prevents inner membrane targeting and insertion of transmembrane segments that are sufficiently hydrophobic for signal recognition particle-dependent targeting. These results suggest that a transmembrane domain-specific co-translational targeting mechanism by type III secretion system chaperones prevents co-translational mistargeting by the signal recognition particle for subsequent post-translational secretion of transmembrane domain-containing type III secretion system substrates. Altogether, our results elucidate the fine discrimination between competing targeting pathways that is critical for the virulence of many bacterial pathogens.

Presentation: Tuesday, 7 March 2017 from 8:30 - 8:45 in room 5.

119/MPV
Pathometabolism of Listeria monocytogenes
T. M. Fuchs*[1], W. Eisenreich[2]
[1]*Friedrich-Loeffler-Insitut, Institut für Molekulare Pathogenese, Jena, Germany*
[2]*Technische Universität München, Lehrstuhl für Biochemie, Garching, Germany*

L. monocytogenes is a Gram-positive pathogen that mainly affects immunocompromised individuals, pregnant women and newborns. The most common vehicles of infection by this saprophytic bacterium are dairy products and other foods including eggs, seafood, and vegetables. 659 documented cases of listeriosis were reported for 2015 in Germany (Robert Koch-Institut, EB 2016). The high lethality rate of up to 10% urged the efforts to understand listerial pathogenicity and to combat this pathogen (Vázquez-Boland *et al.*, 2001). A hallmark of this facultatively intracellular pathogen is its capability to escape from the intracellular vacuole by disrupting the phagosomal membrane and to replicate within the cytoplasm of host cells. The metabolic capabilities of *L. monocytogenes* that contribute to proliferation during infection have been deciphered only recently. Two transcriptomic approaches using macrophage and epithelial cells showed that the pentose phosphate cycle, but not glycolysis is the predominant pathway of listerial metabolism in the host environment (Joseph *et al*., 2006; Chatterjee *et al*., 2006). In a pilot study, 13C-isotopologue profiling was established to directly analyze the carbon metabolism of intracellularly *L. monocytogenes* (Eylert *et al.*, 2008). Major findings were that (i) a majority of amino acids can be provided by the host cell, (ii) alanine, aspartate and glutamate are *de novo* biosynthesized, and (iii) C3 metabolites serve as a predominant carbon source for intracellulary replicating listeriae.
Much less, however, was known about the *in vitro* and *in vivo* nitrogen metabolism of *L. monocytogenes*, and we therefore established 15N-isotopologue profiling by which we confirmed glutamine and ammonium as preferred N-sources. Further findings based on the differential 15N-profiles of amino acids are that *L. monocytogens* utilized the branch-chained amino acids valine, leucine, and isoleucine for anabolic purposes, whereas arginine, histidine and cysteine were directly incorporated into proteins. Methionine, ethanolamine and glucosamine were identified for the first time as amino donors that feed the core N-

metabolism of *L. monocytogenes*. Ethanolamine derived from phospholipids by the activity of listerial phospholipases thus may serve as nitrogen, carbon and energy source for pathogens in different habitats including food and the gut (Kutzner *et al*., 2016). The nematode *Caenorhabditis elegans* was then used as a model to study the listerial N-metabolism during colonization of the worm. Interestingly, we observed a flux of N-atoms from the nematode to *L. monocytogenes*, indicating that approximately 10% of the nitrogen sources consumed by listeriae during colonization are host-derived (Kern *et al*., 2016).
We conclude that the pathometabolism, e.g. the metabolic host-pathogen interdependencies, significantly contributes to the infection process.

Presentation: Tuesday, 7 March 2017 from 8:45 - 9:00 in room 5.

120/MPV

Structures of two bacterial resistance factors mediating tRNA-dependent aminoacylation of phosphatidylglycerol with lysine or alanine

S. Hebecker*[1], J. Krausze[2], T. Hasenkampf[1], J. Schneider[1], M. Groenewold[2], J. Reichelt[2], D. Jahn[1], D. W. Heinz[2], J. Moser[1]
[1]TU Braunschweig, Department of Microbiology, Braunschweig, Germany
[2] Helmholtz Centre for Infection Research, Department of Molecular Structural Biology, Braunschweig, Germany

The cytoplasmic membrane is probably the most important physical barrier between microbes and the surrounding habitat. Aminoacylation of the polar head group of the phospholipid phosphatidylglycerol (PG) catalyzed by Ala-tRNA^{Ala}–dependent alanyl-phosphatidylglycerol synthase (A-PGS) or by Lys-tRNA^{Lys}–dependent lysyl-phosphatidylglycerol synthase (L-PGS) enables bacteria to cope with cationic peptides that are harmful to the integrity of the cell membrane. Accordingly, these synthases also have been designated as multiple peptide resistance factors (MprF). They consist of a separable C-terminal catalytic domain and an N-terminal transmembrane flippase domain. Here we present the X-ray crystallographic structure of the catalytic domain of A-PGS from the opportunistic human pathogen *Pseudomonas aeruginosa*. In parallel, the structure of the related lysyl-phosphatidylglycerol–specific L-PGS domain from *Bacillus licheniformis* in complex with the substrate analog L-lysine amide is presented. Both proteins reveal a continuous tunnel that allows the hydrophobic lipid substrate PG and the polar aminoacyl-tRNA substrate to access the catalytic site from opposite directions. Substrate recognition of A-PGS versus L-PGS was investigated using misacylated tRNA variants. The structural work presented here in combination with biochemical experiments using artificial tRNA or artificial lipid substrates reveals the tRNA acceptor stem, the aminoacyl moiety, and the polar head group of PG as the main determinants for substrate recognition. A mutagenesis approach yielded the complementary amino acid determinants of tRNA interaction. These results have broad implications for the design of L-PGS and A-PGS inhibitors that could render microbial pathogens more susceptible to antimicrobial compounds.

Presentation: Tuesday, 7 March 2017 from 9:00 - 9:15 in room 5.

121/MPV

Identification of an integrin as the cellular receptor for PavB of *Streptococcus pneumoniae*

S. Kanwal*[1], V. Reis[2], M. Keller[2], S. Hammerschmidt[1,2]
[1]Interfaculty Institute of Genetics and Functional Genomics, Greifswald, Germany
[2]Institute for novel and emerging infectious diseases, Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Greifswald - Insel Riems, Germany

Introduction: Pneumococcal adherence and virulence factor B (PavB) acts as an adhesin and virulence factor of *Streptococcus pneumoniae*[1]. The major part of the mature PavB consists of repetitive sequences referred to as **S**treptococcal **S**urface **Re**peats (SSURE). PavB interacts in a dual fashion with eukaryotic cells either by acting as an MSCRAMM binding to various matrix proteins[2] or by interacting directly with a yet unknown cellular receptor. In this study we identified an integrin as a potential cellular receptor for PavB.
Methods: *In vitro* cell culture infection assays were carried out to analyze the role of PavB in adherence to and invasion of pneumococci into human epithelial cells. To assess the direct interaction of PavB with epithelial cells, recombinant SSURE domains were labeled with a fluorescent dye and binding to A549 cells was monitored by flow cytometry. Blocking assays were performed with integrin specific monoclonal antibodies. Mouse embryonic fibroblasts expressing or lacking specific integrin subunits were further used to decipher the role of various integrins as cellular receptor for PavB. The direct interaction between PavB and integrins was tested by surface plasmon resonance (SPR) studies.
Results: Flow cytometric analysis indicated a direct interaction of PavB with eukaryotic cells. Strikingly, human vitronectin competitively inhibited PavB binding to nasopharyngeal epithelial cells. This suggested bindinig of PavB to the major vitronectin receptor $\alpha_v\beta_3$ integrin. Functional blocking assays with monoclonal antibodies strengthen the hypothesis that PavB employs $\alpha_v\beta_3$ integrin as its direct receptor on eukaryotic cells. We further confirmed the results in direct binding assays with mouse embryonic fibroblasts where cells lacking $\alpha_v\beta_3$ demonstrated a marked decrease in binding to PavB. Finally, our SPR analysis indicated a direct interaction between pneumococcal PavB and $\alpha_v\beta_3$ integrin.
Conclusions: Pneumococcal PavB interacts directly with host eukaryotic cells. We report here for the first time that pneumococci are able to interact directly with one of the integrins, namely with the $\alpha_v\beta_3$ integrin. This is a new aspect of the versatile interactions of pneumococcal adhesins with eukaryotic receptors.

References

[1] Binsker, U. *et al.* Pneumococcal Adhesins PavB and PspC Are Important for the Interplay with Human Thrombospondin-1. *J Biol Chem* 290, 14542-14555, doi:10.1074/jbc.M114.623876 (2015).
[2] Jensch, I. *et al.* PavB is a surface-exposed adhesin of Streptococcus pneumoniae contributing to nasopharyngeal colonization and airways infections. *Mol Microbiol* **77**, 22-43, doi:10.1111/j.1365-2958.2010.07189.x (2010).

Presentation: Tuesday, 7 March 2017 from 9:15 - 9:30 in room 5.

122/MPV

A novel mechanism of inactivating antibacterial nitro compounds in the human pathogen *Staphylococcus aureus* by overexpression of a flavin-dependent nitroreductase

E. El-Hossary[1], P. Francoise[2], K. Förstner[3], K. Streker[3], J. Schrenzel[2], U. Holzgrabe[4], K. Ohlsen*[3]
[1]*National Centre for Radiation Research & Technology, Cairo, Egypt*
[2]*Geneva University Hospital, Geneva, Switzerland*
[3]*Institut für Molekulare Infektionsbiologie, Würzburg, Germany*
[4]*Institute for Pharmacy, Würzburg, Germany*

Staphylococcus aureus is a leading cause of nosocomial as well as community-acquired infections which are often difficult to treat due to multiple antibiotic resistant strains. In consequence, novel drugs are necessary especially based on new chemical classes and with alternative mode of action compared to the most common antibacterials. We have recently described the synthesis and antimicrobial activity of several bisnaphthalimides. The bisquaternary nitro-substituted bisnaphthalimide MT02 showed high activity against antibiotic resistant *S. aureus* including MRSA. Structure-activity relationship studies revealed that the two nitro groups are essential for high activity. Since MT02 is a promising compound against multi-resistant staphylococci we were interested in selecting MT02-resistant variants. Surprisingly, adaptation to higher MT02 concentrations was linked to the production of a red colour. We determined the chemical nature of the red colour and deciphered the molecular events leading to MT02 resistance. Chromatographic and spectroscopic investigations revealed a stepwise reduction of the nitro groups to amino groups. The corresponding derivatives were completely inactive. We found further by RNAseq analysis that MT02 resistant strains strongly upregulate a novel nitroreductase. Further genetic and biochemical analysis revealed that this novel nitroreductase is responsible for the chemical modification of the nitro-groups of MT02 leading to resistance. Overall, we have identified a novel nitroreductase-based antibiotic resistance mechanism in the human pathogen *S. aureus*.

Presentation: Tuesday, 7 March 2017 from 9:30 - 9:45 in room 5.

123/MPV

The Type IV Effector CaeB from *Coxiella burnetii* functions as a cell death suppressor across kingdoms and modulates ER stress signalling

A. Friedrich*[1,2], S. Sonnewald[1], A. Lührmann[2]
[1]*FAU Erlangen-Nürnberg, Biologie, Erlangen, Germany*
[2]*Universitätsklinikum Erlangen, Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Erlangen, Germany*

Coxiella burnetii is a Gram negative, obligate intracellular zoonotic pathogen that causes Q-fever. Like many other pathogens *C. burnetii* exert control on processes that regulate host cell death, since abrogation of host cell death allows intracellular replication.
C. burnetii possesses a type IV secretion system (T4SS) to deliver about 150 effector proteins into the host cell. The T4SS is essential for intracellular survival and replication. However for only very few secreted T4SS effector proteins a function has been assigned. Three effector proteins, AnkG, CaeA and CaeB, suppress host cell apoptosis, a programmed cell death (PCD) pathway. PCD is crucial for the removal of damaged or infected cells and is thereby an important arm of the innate immune system. The induction of PCD is a genetically controlled and conserved process in eukaryotic cells and results from a complex interaction of microbial proteins with cellular host proteins. The aim of our work is to increase the knowledge about how effector proteins mediate cell death suppression and to investigate to which levels the processes are conserved cross kingdom.
Previously, we have shown that CaeB inhibit cell death in mammalian cells. Here, we demonstrate that CaeB also suppresses cell death in plants. Thus, CaeB inhibits cell death induced by mammalian Bax and also by the interaction of the tomato kinase *Pto* with the corresponding virulence gene *AvrPto*. GFP tagged CaeB localises to the ER *in planta* and in mammalian cells. As ER homeostasis plays an important role in host cell viability, we analysed the role of CaeB in ER stress signalling. Our results demonstrate that CaeB suppresses cell death induced by severe ER stress *in planta* as well as in the mammalian system. Interestingly, CaeB seems to modulate the activity of the ER stress sensor inositol-requiring enzyme (IRE) 1a. Thus, CaeB expression results in activation of IRE1a-mediated endoribonuclease activity, as presented by accelerated splicing of XBP-1 (in mammalian cells) and of b-ZIP60 (*in planta*). Additionally, CaeB inhibits IRE1a-mediated protein kinase activity, as demonstrated by reduced phosphorylation of p38 in GFP-CaeB expressing mammalian cells. Taken together CaeB is cross-kingdom cell death suppressor, which might inhibit cell death by shifting the activity of the ER stress sensor IRE1a.

Presentation: Tuesday, 7 March 2017 from 9:45 - 10:00 in room 5.

WORKSHOP 21
Gut microbial diversity, diet, and health (FG PW)
07 March 2017 • 08:30 – 10:00

124/PWV

Analysis of the active microbiome along the gastrointestinal tract (GIT) of pigs using metaproteomics

J. B. Tröscher[1], B. Tilocca[1], J. Seifert*[1]
[1]*University of Hohenheim, Animal Science, Stuttgart, Germany*

Question: Pigs belong to the most important farm animals worldwide and it is therefore of great interest to characterize their intestinal microbiome for an improvement of animal health and feed conversion. Besides the agricultural interests, pigs are useful animal models for human-related health studies. Similarities are the omnivorous lifestyle and the anatomy in general and that of the GIT. Since the microbial composition is essentially modulated by the diet, the digesta is of interest for diet-related questions. In addition, the mucosa acts as the key interface between the host and the microbiome hosting specific microbial species. Thus, digesta and mucosa samples from different sections of the porcine GIT were analyzed by metaproteomics to obtain a deeper insight into the functions of bacterial groups.
Methods: Four pigs were chosen to dissect the stomach, jejunum, ileum, cecum and colon and to take digesta and mucosal samples out of these sections. The samples were separately treated to remove feed residues and eukaryotic cells. The obtained bacterial cell pellets were lysed by using sonication, temperature, and chemicals. Extracted proteins were purified with a 1D-SDS gel and used for in-gel trypsin digestion. Purified peptides were analysed using LC-ESI-MS/MS (Thermo Scientific Q Exactive plus system). The MS/MS data were used to infer protein IDs through MaxQuant and perform further examination using Unipept and WebMGA for phylogenetic and functional analyses.
Results: In total, 2951 bacterial proteins were identified with a higher number of bacterial proteins recovered in the digesta (2917) than in the mucosa (973). The phylogenetic affiliation of the proteins showed different characteristics between the samples. Proteins of Prevotellaceae (Bacteroidetes) dominated mucosal samples, whereas proteins of Clostridiaceae and Lactobacillaceae (Firmicutes) were the highest represented in digesta samples. Proteins of Proteobacteria were considerably lower on mucosal site. Main functions of proteins such as energy production and conversion, translational functions and carbohydrate transport and metabolism were the same in mucosa and digesta. Proteins of

cecum and colon samples showed higher phylogenetic and functional diversities compared to the stomach and small intestine. Protein functions were more homogenous along sections in the digesta than within the mucosa.
In addition, pig proteins were co-extracted and measured. In total, 4550 porcine proteins were identified. Their classification showed clear separations based on the different gut locations.
Conclusions: The study produced a wealth of information about the microbial and intestinal biology of the pig. For the first time, the active fraction of the porcine microbiome along the whole GIT was studied, highlighting a first insight into the functional distribution of the intestinal microbiome.

Presentation: Tuesday, 7 March 2017 from 8:30 - 8:45 in room 12.

125/PWV

Probiotic and prebiotic effects in the caeca microbial metagenome of laying hens

D. Borda Molina*[1], C. Pineda Quiroga[2], D. Chaves Moreno[3], R. Ruiz[2], R. Atxaerandio[2], A. Camarinha Silva[1], A. García Rodriguez[2]
[1]*University of Hohenheim, Institute of Animal Science, Stuttgart, Germany*
[2]*Neiker-Tecnalia , Department of animal production, Vitoria-Gasteiz, Spain*
[3]*Helmholtz Centre for Infection Research, Braunschweig, Germany*

Question: Diets play an important role in modulating caeca microbial community. Studies have been focused in safe additives such probiotics and prebiotics and its dual effect, to improve animal health and performance. To assess the effect of supplementing corn-soybean diets of laying hens with dry whey powder (DWP) as a prebiotic, *Pediococcus acidilactici* as a probiotic (PA) and the combination of both (DWP-PA) (synbiotic), Illumina amplicon sequencing and whole metagenome sequencing were used to explore microbial variations.
Methods: A total of 300 laying hens, 57 weeks of age, were randomly allocated to floor pens for 70 days. Pens were assigned to four experimental diets. The treatments included a control diet, DWP diet (60 g/kg of DWP), PA diet (20 g/kg of PA), and DWP-PA diet (mixture of 60 g/kg of DWP and 20 g/kg of PA). Caecal contents were taken from 12 individual hens to proceed with DNA extractions and Illumina amplicon sequencing procedure, targeting the 16S rRNA V1-2 region1. Metagenomes of four individual samples, corresponding to each treatment, were sequenced through Illumina HiSeq2500 platform.
Results: A clear grouping of the samples per diet was observed ($p < 0.05$). Ruminococcaceae was detected in similar abundance in all treatments (12%). Bacteroidaceae, Prevotellaceae and Porphyromonadaceae were the most abundant families in the control and synbiotic diet, with abundances between 5 to 19%. In synbiotic diet Coriobacteraceae and Lachnospiraceae were present in higher abundances in comparison to the other dietary treatments. Samples PA diet showed lower abundance of Lactobacillaceae. The addition of DPW to the diet promotes the presence of *Olsenella*, known to be a fermenter of carbohydrates and to produce predominantly lactic acid2. *Lactobacillus crispatus* was more abundant in DWP diet. In regards to the metagenome analysis, circa 30% of the reads could not be taxonomically assigned. A core of main functions in the four metagenomes was identified (1204). Specific genes encoding for calcium mediated signalling and fatty acid synthase activity were only observed in DWP diet, while probiotic treatment revealed genes encoding for phosphatase activities regulation. Alpha amylase and chitinase activities were only found in the DWP-PA sample.
Conclusion: The influence of the dietary supplementation in the total caecal microbial community does not imply a disturbance in the main metabolic roles. Specific functions, encoded by the community, are present or absent depending on the source of supplementation.

References

[1] Camarinha-Silva et al., Environmental Microbiology, 2014, 16:2939-2952.
[2] Kraatz et al., International Journal of Systematic and Evolutionary Microbiology, 2011, 61:795-803.

Presentation: Tuesday, 7 March 2017 from 8:45 - 9:00 in room 12.

126/PWV

Gut Bacteria of the Family *Coriobacteriaceae* increase white adipose tissue deposition in mice

S. Just*[1], K. Wegner[2], S. Mondot[3], S. Theresa[1], G. Laura[2], C. Philippe[4], G. Héry-Arnaud[3], J. Ecker[5], P. Gerard[4], S. Rohn[2], P. Lepage[3], T. Clavel[1]
[1]*Technical University of Munich, ZIEL Institute for Food and Health, Freising, Germany*
[2]*Hamburg School of Food Science, Department for Food Chemistry , Hamburg, Germany*
[3]*INRA, MICALIS Institute - PhylHom, Jouy-en-Josas, France*
[4]*INRA, MICALIS Institute - AMIPEM, Jouy-en-Josas, France*
[5]*Technical University of Munich, Chair for Nutritional Physiology, Freising, Germany*

Introduction: *Coriobacteriaceae (CORIO)* are dominant members of the human gut microbiota and can convert cholesterol-derived metabolites such as bile acids. However, consequences for the host are unknown. The aim of the present study was to characterize the effects of *CORIO* on metabolic health *in vivo*.
Methods: Male germ-free (GF) C57BL/6N mice were associated at week 5 of age with a consortium of four *CORIO* strains. At week 10, mice were randomly divided into 3 groups (n = 12 each) fed a control (CD), high-fat (HFD), or bile acid-supplemented (BA) diet for 16 weeks. GF and specific-pathogen free (SPF) mice were used as controls.
Results: Characterization of the bacteria *in vitro* revealed bile salt hydrolase, hydroxysteroid dehydrogenase, and lipase activities. In mice, HFD increased body weight and induced steatosis, independent of the colonization status. Strikingly, all mice fed the BA diet stayed lean, but those colonized with *CORIO* had 75 to 150 % more white adipose tissue (WAT) (GF, 37 ± 15; *CORIO*, 65 ± 15, SPF, 26 ± 16 mg/ g body weight, $p<0.001$). No difference in adipocyte size was observed, suggesting that hyperplasia rather than hypertrophy was responsible for the phenotype. Proteome analysis of epididymal WAT revealed several *CORIO* mice-specific proteins related to lipid metabolism, including CIDEC, a protein involved in fatty acid trafficking in lipid droplets. In the liver, *CORIO* mice fed BA diet showed increased amounts of total and monounsaturated fatty acids (FA), whereas polyunsaturated FA amounts were lower, reflecting altered lipid metabolism. Modulation of bile acid metabolism was indicated by increased expression of FXR and Cyp7a1 in the gut and liver of *CORIO* mice, but only minor differences in bile acid concentrations in various body fluids were detectable. The increase in WAT observed in *CORIO* mice fed the BA diet was associated with signs of metabolic disturbances, including increased systemic insulin (GF, 0.8 ± 0.3; *CORIO*, 1.8 ± 1.0; SPF, 1.2 ± 1.0 ng/ ml; $p < 0.05$) and leptin levels (GF, 3.3 ± 2.9; *CORIO*, 11.5 ± 8.0; SPF, 2.0 ± 1.4 ng/ ml; $p < 0.001$). These changes were accompanied by systemic hypercholesterolemia (GF, 92 ± 40; *CORIO*, 141 ± 40; SPF, 56 ± 23 µM; $p < 0.05$), which was also observed in *CORIO* mice fed CD and HFD. Metagenomic, functional genomic, lipidomic, and additional mouse experiments are underway to characterize molecular mechanisms underlying the effects of *CORIO*.
Conclusion: Interactions between *Coriobacteriaceae* and bile acids influence fat tissue deposition, cholesterol, and lipid metabolism in mice, providing novel insights into the role of specific gut bacteria in regulating host metabolism.

Presentation: Tuesday, 7 March 2017 from 9:00 - 9:15 in room 12.

127/PWV

Gut microbial glycerol metabolism with potential implications on host health

C. Schwab*[1], C. Engels[1], H. J. Ruscheweyh[1], J. Zhang[1], S. Sturla[1], C. Lacroix[1]
[1]*ETH Zurich, Zurich, Switzerland*

In the gut, glycerol becomes available as a product of luminal microbial fermentations, digestion of luminal fats, sloughed mucus and desquamated epithelial cells, and intestinal clearing of endogenous plasma. Three major microbial pathways to degrade glycerol exist. Glycerol kinase and glycerol-3-phosphate dehydrogenase, or glycerol dehydrogenase and dihydroxyacetone kinase activity yield dihydroxyacetone phosphate which is an intermediate of glycolysis. Cobalamin-dependent glycerol/diol dehydratases PduCDE catalyze the transformation of glycerol to 3-hydroxypropionaldehyde (3-HPA), a component of the reuterin system. Reuterin has antimicrobial properties and undergoes chemical conjugation with the food-derived heterocyclic amine 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine (PhIP), which is implied in the development of colorectal cancer. In aqueous solution reuterin is in dynamic equilibrium with the toxicant acrolein, while the role of acrolein in activities attributed to reuterin has been little studied.
It was the aim of this study to (1) specify the HCA binding activities of the different components of the reuterin system, to (2) identify gut microbes with glycerol/diol dehydratase activity, and to (3) estimate the potential of gut microbial communities to metabolize glycerol by glycerol/diol dehydratase.
The application of a combined novel analytical approach including IC-PAD, LC-MS and NMR together with specific acrolein scavengers revealed for the first time that acrolein and not 3-HPA is responsible for HCA conjugation.
The frequently occurring gut microbe *Eubacterium hallii* was identified harboring a cobalamin-dependent glycerol/diol dehydratase beside other gut taxons, such as *Ruminococcus obeum*, *Ruminococcus gnavus*, *Flavonifractor prautii*, *Intestinimonas butyriciproducens*, and *Veillonella* spp. *E. hallii* metabolized glycerol to a maximum of 9 mM 3-HPA while acrolein formed from 3-HPA interacted with PhIP to produce the conjugate PhIP-M1.
Fecal metagenomes were screened for the presence of glycerol-utilization pathways. Genes potentially coding for glycerol kinase/glycerol-3-phosphate dehydrogenase, and glycerol dehydrogenase/dihydroxyacetone kinase were more often detected than those for glycerol/diol dehydratase. All metagenomes harbored glycerol/diol dehydratase encoding genes, while *E. hallii* PduCDE was detected in 63 to 81% of the metagenomes (n=152) depending on which subunit was queried.
Our findings indicate the potential of glycerol/diol dehydratase activity of gut microbes in the transformation of PhIP to the less mutagenic PhIP-M1. Acrolein-dependent transformation of HCAs might lead to detoxification of dietary carcinogens with possible health impact related to the development of colorectal cancer.

Presentation: Tuesday, 7 March 2017 from 9:15 - 9:30 in room 12.

128/PWV

Characterization of a type VII secretion system in *Staphylococcus epidermidis* and its possible role in host evasion

S. B. Lassen[1], R. L. Meyer[2], H. Brüggemann*[1]
[1]*Aarhus University, Biomedicine, Aarhus, Denmark*
[2]*Aarhus University, Bioscience, Aarhus, Denmark*

A type VII secretion (T7S)-like system has recently been discovered in a strain of the human skin-associated species *Staphylococcus epidermidis* (1). This strain has a high antimicrobial activity against other skin organisms such as *Propionibacterium acnes*. The T7S gene locus is linked to a set of genes encoding polymorphic (PM) toxins with nuclease activity, and it was predicted that secretion of these nuclease toxins via T7S might be responsible for the profound antimicrobial activity of this strain (1). A T7S system-secreted nuclease toxin has recently been identified in *Staphylococcus aureus* (2).
Here, the dissemination of the T7S locus in the *S. epidermidis* population was investigated. It was revealed that the T7S locus is restricted to strains that belong to a distinct phylogenetic clade consisting of health-associated human, mouse and plant isolates. Whereas the T7S gene locus was conserved, the PM toxin locus showed extended strain-specific variations.
In order to study the function of the T7S system, a knock-out mutant strain was constructed that lacked the essential EssC structural component. The mutant exhibited a similar antimicrobial phenotype as the wildtype strain. However, in contrast to the wildtype strain, the mutant produced a biofilm matrix, indicating that a T7S substrate might be involved in restricting biofilm formation. We propose that secreted PM toxins with nuclease and/or protease activities are responsible for disrupting biofilm matrix components.
Furthermore, in cell culture experiments it was shown that the T7S system is involved in modulating host immunity: co-incubation of the mutant strain with keratinocytes resulted in increased interleukin-8 production as compared to co-incubation with the wildtype strain.
Taken together, our data show that the T7S system in *S. epidermidis* is functional and has roles in modulating colonization and host immunity.

References

[1] Christensen GJ, Scholz CF, Enghild J, Rohde H, Kilian M, Thürmer A, Brzuszkiewicz E, Lomholt HB, Brüggemann H. Antagonism between *Staphylococcus epidermidis* and *Propionibacterium acnes* and its genomic basis. BMC Genomics 17:152 (2016).
[2] Cao Z, Casabona MG, Kneuper H, Chalmers JD, Palmer T. The type VII secretion system of *Staphylococcus aureus* secretes a nuclease toxin that targets competitor bacteria. Nat Microbiol 2:16183 (2016).

Presentation: Tuesday, 7 March 2017 from 9:30 - 9:45 in room 12.

129/PWV

Antibiotic-mediated alterations in the intestinal microbiota affect large intestinal pancreatic protease activity and colitogenic mechanisms in IL10-/- mice

H. Yoon*[1], M. Schaubeck[2], D. Haller[1], G. Hörmannsperger[1]
[1]*Technische Universität München, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany*
[2]*Max Planck Institute of Neurobiology, Department of Neuroimmunology, Munich, Germany*

Introduction: Antibiotic (AB) therapy is associated with increased risk for the development of inflammatory bowel diseases (IBD) but the colitogenic role of ABs is not well understood. It has been shown that several ABs, due to AB-specific eradication of bacterial commensals, mediate a strong rise

in large intestinal luminal protease activity (liPA) in rodents, but the pathophysiological relevance of this adverse effect is unclear.
Objectives: Our aim is to reveal detrimental effects of AB-mediated increase in liPA on the intestinal barrier and the development of chronic intestinal inflammation.
Methods: We analyzed the liPA in patient stool and gut contents of untreated, vancomycin/metronidazole (V/M)-treated and germfree (GF) wildtype (WT) and IL10-/- mice using protease assays and LC-MS/MS analysis. Intestinal barrier functions were measured using transwell and Ussing chamber analyses. Acute (2d) and pulsed AB therapies (7d, at 4/8 weeks of age) were used to investigate liPA, barrier function and colitis development in IL10-/- mice.
Results: Analysis of stool samples before and during AB therapy revealed a more than 5fold increase in liPA in about 25% of patients. In mice, V/M treatment resulted in a rapid increase in liPA (5-10fold), which was mostly linked to pancreatic trypsin. The abnormally high liPA was found to impair the intestinal epithelial barrier *in vitro* and *ex vivo*. In WT and IL10-/- mice, the acute V/M-mediated rise in liPA resulted in increased permeability of the intestinal barrier *in vivo* and impaired large intestinal barrier functions *ex vivo*. Co-administration of a serine protease inhibitor reversed these detrimental effects. Pulsed AB treatment resulted in a long lasting increase in liPA and accelerated colitis development in IL10-/- mice. From the age of 14 weeks on, V/M-treated IL10-/- mice showed significantly increased serum amyloid A and fecal complement C3 levels compared to untreated mice. At 16 weeks, V/M-treated IL10-/- mice show significantly increased histopathological inflammation and large intestinal tumor formation.
Conclusion: The V/M-mediated rapid increase in liPA impairs the intestinal barrier in healthy and colitis susceptible mice and is associated with accelerated development of chronic inflammation in colitis susceptible mice. These findings suggest that specific AB therapies may causally contribute to the development of IBD in susceptible individuals.

Presentation: Tuesday, 7 March 2017 from 9:45 - 10:00 in room 12.

WORKSHOP 23
Nosocomial Infectionss and Outbreaks: Epidemiology, diagnostic, surveillance and Prevention (FG PR / StAG HY)
07 March 2017 • 08:30 – 10:00

130/HYV
Seroprevalences of Antibodies against Measles, Rubella and Varicella among Asylum Seekers Arriving in Lower Saxony, Germany, November 2014–October 2015

J. Dreesman*[1], S. E. Toikkanen[1,2], E. Mertens[1], A. Baillot[1]
[1]*Niedersächsisches Landesgesundheitsamt, Hannover, Germany*
[2]*European Programme for Intervention Epidemiology Training (EPIET), European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden*

Question: According to official numbers, about 100.000 asylum seekers arrived in Lower Saxony in 2015, meaning an enormous challenge for provision of accommodation. Overcrowding in reception centres increases the risk for spread of infections. Thus specific measures of infection control were required. In Lower Saxony, arriving asylum seekers 12 years and older were screened for antibodies against measles, rubella and varicella (MRV) from November 2014 to October 2015 to support such measures. We analysed the data to detect immunisation gaps.
Methods: Serum samples were tested by measles and varicella zoster virus-ELISA IgG (Sukisui Virotech GmbH, Rüsselsheim, Germany) and rubella virus SERION ELISA classic IgG (Virion/Serion GmbH, Würzburg, Germany) for the presence of IgG antibodies . Seroprevalences were calculated by disease and country of origin with 95% confidence intervals (95%CI). A group was defined sufficiently protected if the seroprevalence exceeded a given herd immunity threshold (95% for measles and rubella, 91% for varicella) statistically significantly. Logistic regression models were fitted to examine whether sex or age were associated with being seropositive after adjustment for country of origin.
Results: 23,647 serum samples were included. Median age of participants was 26 years, 75.6% were male and 62 countries were reported. The overall seroprevalence was 79.9% for measles, 85.1% for rubella and 87.5% for varicella; all below the thresholds. Stratification by country of origin showed that no groups, except asylum seekers from Syria against varicella (93.4%, 95%CI 92.6%–94.0%), were sufficiently protected against MRV. One year increase in age significantly increased the odds for being seropositive for MRV by 3-6%. Males were 1.32 times more likely rubella-seropositive than females.
Conclusions: Utilisation of the data can aid public health institutions to prioritize vaccinations in reception centres to groups with lowest seroprevalences if not every asylum seeker can be vaccinated. In Lower Saxony this has already been shown to be successful in varicella outbreak management.
Publication: doi:10.3390/ijerph13070650,
http://www.mdpi.com/1660-4601/13/7/650

Presentation: Tuesday, 7 March 2017 from 8:30 - 8:45 in room 6.

131/HYV
Management of an outbreak with OXA-48 producing Klebsiella pneumoniae in a university hospital in Germany

T. Artelt*[1], I. Bley[1], I. Thalmann[1], H. Eiffert[2], U. Reichard[3], Y. Pfeifer[4], M. Kaase[1], S. Scheithauer[1]
[1]*University Medicine Göttingen UMG, Infection Control and Infectious Diseases, Göttingen, Germany*
[2]*University Medicine Göttingen UMG, Institut für Medizinische Mikrobiologie, Göttingen, Germany*
[3]*Amedes Group, Göttingen, Germany*
[4]*Robert Koch-Institut, Wernigerode, Germany*

Introduction: Outbreaks caused by carbapenemase-producing Enterobacteriaceae are of major importance in a hospital due to the risk of life-threatening infections with only limited treatment options. The most targeted interventions to control such outbreaks are still debated.
Objectives: To evaluate the efficiency of extensive measures to control an outbreak with OXA-48 producing Klebsiella pneumoniae with main focus on screening for asymptomatic carriers.
Patients and Methods: Detection of OXA-48 producing K. pneumoniae isolates in clinical cultures of two patients prompted screening for asymptomatic carriers in contact patients by rectal swab culture with use of selective chromogenic agar media. In the initial phase only patients with direct contact defined as patients with stay in the same room as patients with OXA-48 producing K. pneumoniae were screened once. In a later phase screening was extended to patients with indirect contact defined as stay on the same ward as patients with OXA-48 producing K. pneumoniae thrice weekly. Strain typing was performed by pulsed-field gel electrophoresis (PFGE).
Results: Between April and August 2014 an outbreak of patients with OXA-48 producing K. pneumoniae was observed on intensive or intermediate care units in a German university hospital. A total of 47 direct contacts and 717 indirect contacts were identified and screened for carriage of carbapenemase-producing Enterobacteriacea. Among these direct and indirect contacts further patients with OXA-48 producing K. pneumoniae were found in 5 (10.6%) and 1 (0.1%) cases, respectively. Apart from one patient with an infection, the others were classified as

being colonized. Hospitalization abroad as risk factor was not found in any of the eight patients. However, one patient found by screening was admitted from a long-time care facility, that was implied in another introduction of OXA-48 producing K. pneumoniae into the university hospital in the previous year. This patient had a previous stay in our hospital immediately before the first case of OXA-48 producing K. pneumoniae in 2014. PFGE revealed that all isolates belong to the same clone.
Conclusions: This study demonstrates the huge impact of screening for asymptomatic carriers of carbapenemase-producing Enterobacteriaceae in an outbreak setting. Careful analysis of the patient histories suggests that a patient referred from a long-time care facility was the most likely index patient of this outbreak. This highlights that not only patients with previous hospitalization abroad might be at risk for carriage of carbapenemase-producing Enterobacteriaceae.

Presentation: Tuesday, 7 March 2017 from 8:45 - 9:00 in room 6.

132/PRV
Adaption of hygiene measures after extensive surveillance for multi-drug resistant bacteria in refugee patients
A. Kossow*[1], B. Stuehmer[2], S. Willems[1], S. Kampmeier[1], F. Schaumburg[3], K. Becker[3], B. Glatz[4], M. Möllers[4], A. Mellmann[1]
[1]*University Hospital Muenster, Institute of Hygiene, Muenster, Germany*
[2]*University Hospital Muenster, International Patient Management, Muenster, Germany*
[3]*University Hospital Muenster, Institute for Medical Microbiology, Muenster, Germany*
[4]*University Hospital Muenster, Clinic for Gynecology and Obstetrics, Muenster, Germany*

Background: In 2015 a high number of refugees arrived in Germany to seek asylum. Alongside a number of challenges for the German society, the health system was confronted with patients of whom little medical data exist. Considering the assumed high prevalence of multi-drug resistant bacteria in countries of origin, health care providers had to decide upon possible infection control measures. Infection control specialists at the University Hospital Muenster decided for a relatively restrictive approach: All refugee inpatients were presumptively isolated until carriage of for multi-drug resistant bacteria was excluded via screening. Hygiene measures were adapted after the analysis of the obtained surveillance data.
Materials/Methods: Refugee patients were screened for methicillin-resistant *Staphylococcus aureus* (MRSA), multi-resistant gram-negative bacteria, according to the definition of the Robert-Koch Institute (MRGN), and vancomycin-resistant enterococci (VRE) upon admission. Swabs were taken from nose/throat for MRSA and anally for VRE and MRGN. After phenotypical species identification and antibiotic susceptibility testing, antibiotic resistances were confirmed genotypically comprising *mecA/C* and *vanA/B* genes. Furthermore, all MRSA isolates were characterized by *spa*-typing.
Results: In total, 217 refugee inpatients were prospectively included. Overall, 191 patients were screened for MRSA, 133 for MRGN and 111 for VRE respectively. Of these 9.4% (n=18) were positive for MRSA, 15.0% (n=20) for MRGN and 0.9% (n=1) for VRE. Twelve different MRSA *spa*-types were detected, including t127 (n=4), t223(n=3), t304(n=2), t012, t790, t690, t1627, t034, t311, t4573, t991 and t044 (n=1 each). During the study period, the prevalence of MRSA in all inpatients, screened in the general admission screening, was at 0.8%. The MRGN were all categorized as 3-MRGN belonging 5 different species with *E. coli* being the most common species. Considering the low prevalence of 4-MRGN and VRE in refugee patients, we stopped general pre-emptive isolation of refugee patients after analysing data of 6 months surveillance.
Conclusion: Our 6 month surveillance data showed low prevalence of VRE and 4 MRGN in refugee patients whereas MRSA prevalence was comparatively high. Based on this data it was possible to revaluate our infection control strategy. After future analysis of data obtained during one year, we will re-evaluate the screening strategy for MRGN and VRE of non-risk patients. As a general admission screening for MRSA exists in our institution, carriers of MRSA will still be detected within a reasonable time after admission.

Presentation: Tuesday, 7 March 2017 from 9:00 - 9:15 in room 6.

133/PRV
Antiseptic bathing of intensive care patients – What is the current routine practice in Germany?
L. Denkel*[1], P. Gastmeier[1], R. Leistner[1], C. Geffers[1]
[1]*Institute of Hygiene and Environmental Medicine, Berlin, Germany*

Background: Studies have suggested that antiseptic bathing prevents central line-associated blood stream infections (CLABSI) and acquisition of multi-drug resistant organisms (MDRO) including methicillin resistant *Staphylococcus aureus* (MRSA) in intensive care units (ICUs). An American survey among physician members of the Emerging Infections Network found out that 325 of 354 physicians responding (92 %) used chlorhexidine gluconate bathing for all ICU patients. So far, general antiseptic bathing is no routine practice in German ICUs.
Materials/Methods: ICU-KISS is the German national surveillance system for nosocomial infections in intensive care units (ICU-KISS). ICU-KISS-participants with increased BSI rates (> median, between January 2014 and June 2015) were asked to participate in a survey about the routine washing procedures on their ward distributed by email. This cross-sectional survey was conducted between April and September 2016.
Results: 221 of 350 ICUs (63.1 %) responded to the survey. 134 of 221 respondents (60.1 %) established a standardized protocol for daily patient bathing. 48.5 % (65 of 134) of ICUs with a standardized protocol reported the general use of antiseptic products for all patients. The most frequently applied antiseptics were octenidine (46 ICUs, 70.8 %), polyhexanide (10 ICUs, 15.4 %), chlorhexidine gluconate (3 ICUs, 4.6 %) and others (4 ICUs, 6.2 %). Antiseptic bathing was performed with one-way wash mitts / wipes impregnated with the respective antiseptic in 57 of 65 ICUs (87.7 %). In contrast, ICUs with a non-antiseptic bathing protocol (n = 69) used wash lotions in 87.0 % (n = 60) of cases compared to one-way wash mitts / wipes in 13.0 % (n = 9). In summary, 65 of 221 ICUs included (29.4 %) established a general decolonization protocol for their ward.
Conclusion: General antiseptic bathing including all patients, not only those colonized with MDRO, is increasingly applied among German ICUs. Still, multicenter RCTs are needed to investigate benefits and risks of this infection control measure to provide evidence-based recommendations for its application in German ICUs.

Presentation: Tuesday, 7 March 2017 from 9:15 - 9:30 in room 6.

134/PRV
WGS-based elucidation of linezolid resistance locus *optrA* in clinical *Enterococcus* spp. isolates from Germany
J. Bender*[1], C. Fleige[1], D. Lange[1], U. Geringer[1], I. Klare[1], G. Werner[1]
[1]*Robert Koch Institute, Division 13 Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany*

Introduction: Linezolid (LZD)-resistant *Enterococcus* spp. (LRE) are generally detected at low prevalence throughout Europe. However, the National Reference Centre (NRC) for Staphylococci and Enterococci in Germany has received an increasing number of clinical LRE isolates in recent years. Resistance to this last resort antibiotic can be achieved by either successive accumulation of chromosomal mutations and/or by

acquisition of the methyltransferase Cfr or the recently identified ABC-transporter OptrA. Both genes were described to reside on plasmids or other mobile genetic elements. It has been hypothesized that these elements were co-selected by antibiotic use in livestock and emerged in staphylococci and enterococci from food-producing animals.
Objectives: Little is known about the prevalence of the novel transporter OptrA in clinical *Enterococcus* (*E.*) isolates from Germany. Thus, we aimed to comprehensively asses the prevalence and loci organization of *optrA* in LRE received by the NRC from 2007 until 2016.
Methods: In total, 539 LRE isolates were screened by PCR for the presence of *optrA*. Eighteen *optrA*-positive LRE were subjected to whole genome sequencing (WGS) to infer LZD-mediating genomic mutations, presence of *cfr*, allelic variations and insertion sites of the *optrA* sequence and the phylogenetic relationship of the strains. Localization of *optrA* was additionally examined by S1-PFGE and Southern hybridization.
Results: A high proportion of LZD-resistant *E. faecalis* (on average 32.3 %) were tested positive for *optrA* whilst only 1.4 % of all LZD-resistant *E. faecium* carried the transporter gene. It is worth mentioning that within the collection of isolates from the NRC, and although the relative number of LZD-resistant *E. faecalis* remained low, an increase of *optrA*-positive *E. faecalis* isolates from 0 % to 87.5 % (2007 to 2016) was observed. Multiple genomic mutations as well as different *optrA* sequences were detected. Phylogenetic analysis based on single nucleotide polymorphisms of the core genome clearly demonstrated that multiple introduction events of the resistance locus had occurred and that this was independent of the date or federal state of isolation. Bioinformatics analyses produced plasmid sequences identical to already described *optrA*-containing vectors as well as novel *optrA* insertion sites.
Conclusion: Our analyses suggest that highly plastic *optrA*-encoding mobile genetic elements emerged especially in *E. faecalis* clinical isolates in recent years. This represents a worrisome situation with respect to resistances against last resort antibiotics and demonstrates the One Health dimension of a putative co-selective effect by antibiotic use in either sector. Immediate attention and thorough examination of resistance gene transmission is required in order to prevent further dissemination of multi-drug resistant pathogens.

Presentation: Tuesday, 7 March 2017 from 9:30 - 9:45 in room 6.

135/PRV
The impact of post-discharge suveillance of surgical site infections in caesarian section
P. Schuller[1], A. Wöckel[2], O. Chow[2], U. Vogel*[3,1]
[1]*University Hospital Würzburg, Infection Control Unit, Würzburg, Germany*
[2]*University Hospital of Würzburg, Obstetrics and Gynecology, Würzburg, Germany*
[3]*University of Würzburg, Institute for Hygiene and Microbiology, Würzburg, Germany*

Introduction: Surveillance of surgical site infection is a component of quality control and prevention of healthcare associated infections. Post-discharge surveillance (PDS) increases the sensitivity of case finding. It is, on the other hand, labor intensive and may lack specificity depending on the procedure employed.
Objectives: To assess the contribution of PDS to case finding in the surveillance of SSI after caesarian section (CS).
Methods: Surveillance was conducted at the Würzburg University hospital with appr. 1900 births and 600 CS p.a. The standard protocol of the German nosocomial infection surveillance system (KISS) was employed. A period of 30 days after CS was analyzed irrespective of the date of discharge. PDS was performed using a standardized questionnaire for telephone interviews.
Results: The surveillance period covered 29 months. A total number of 1,235 patients was analyzed. We identified 36 cases with SSI, 78% of which were categorized as class A1 (superficial incisional SSI), 14% as A2 (deep incisional SSI), and 8% as A3 (organ or visceral cavity SSI). Of the total number of 36 cases with SSI, only 5 were observed during the initial hospital stay (15%). PDS revealed 31 cases, of which 22 were independently recognized during a post-discharge visit at the hospital.
Conclusions: The sole assessment of SSI developed during hospital stay underestimated the total number of SSI by 85% and is therefore insufficient for surveillance. In contrast, only 25% of the total number of patients with putative SSI was identified by telephone interview alone. In the setting described here, the benefit of the labor intensive PDS by telephone interview therefore is negligible.

Presentation: Tuesday, 7 March 2017 from 9:45 - 10:00 in room 6.

PLENARY 04
Phages and New Viruses
07 March 2017 • 11:00 – 12:30

136/INV
Giant viruses every year a surprise!
D. Raoult*[1]
[1]*Université Aix-Marseille, Faculté de Médecine, Unité des Rickettsies, Marseille, France*

No abstract has been submitted.

Presentation: Tuesday, 7 March 2017 from 11:00 - 11:30 in the Franconia Hall.

137/INV
Bacterial control of phage predation by bifunctional abortive infection and toxin-antitoxin systems
G. Salmond*[1]
[1] *University of Cambridge, Department of Biochemistry, Cambridge, United Kingdom*

Bacteria are susceptible to infection by their viral predators (bacteriophages; phages) and these viruses are the most abundant biological entities on Earth. Bacteria evolve diverse strategies for evading the lethal impacts of phage infection and, correspondingly, phages evolve to circumvent bacterial defensive systems - an eternal co-evolutionary molecular arms race. Abortive infection (Abi) systems in bacteria are post-infection defence mechanisms that terminate viral morphogenesis through "suicide" of infected cells in a process akin to a prokaryotic apoptosis. In this way, productive phage replication is precociously blocked and so sibling bacteria in a clonal population are not infected. Some Abi systems have Type III toxin-antitoxin (TA) functionality in which a proteinaceous toxic endoribonuclease is functionally suppressed by antitoxic small RNA species in a pseudoknot configuration, within a hetero-hexameric quaternary complex. After infection by some phages the bifunctional Abi/TA system may be "destabilised", releasing the toxic endoribonuclease which leads to eventual death of the infected cell after mRNA degradation. Some phages that induce the Abi response have the capacity to evolve spontaneous viral mutants that "escape" or circumvent the Type III TA system to enable a productive lytic cycle. Analysis of various independent mutants of different phages has revealed that there must be multiple molecular routes to Abi evasion.

Presentation: Tuesday, 7 March 2017 from 11:30 - 12:00 in the Franconia Hall.

138/INV
The Wonderful World of Archaeal Viruses
D. Prangishvili*[1]
[1]*Institut Pasteur, Department of Microbiology, Paris, France*

Viruses infecting Archaea constitute one of the most enigmatic sections of the virosphere. They display remarkable diversity of unexpected, complex morphotypes, not encountered among viruses of Bacteria and Eukarya. Moreover, the overwhelming majority of their genes do not have homologs in the extant databases. The distinctiveness of archaeal viruses extends to the structures of the proteins they encode and the mechanisms underlying their interactions with the host cells.
In my talk I will summarize the state of art of our studies on archaeal viruses and emphasize the impact of these studies on our understanding of the diversity and evolution of the virosphere.

Presentation: Tuesday, 7 March 2017 from 12:00 - 12:30 in the Franconia Hall.

HYGIENESYMPOSIUM 02
Neues zur mikrobiologischen Diagnostik und Qualitätssicherung (FG QD / StAG DV)
07 March 2017 • 11:00 – 12:30

139/HYV
Impact of healthcare-associated infections in Europe: Results of a population prevalence-based modelling study
D. Plachouras*[1]
[1]*European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden*

Background: Estimating the impact of healthcare-associated infections (HAIs) is challenging due to the need for good quality incidence data and the co-morbidities of hospitalised patients. We estimated the burden of six common HAIs using the methodology of the Burden of Communicable Diseases in Europe (BCoDE) project and 2011-2012 data from the ECDC point prevalence survey (PPS) of HAIs.
Methods: The studied HAIs were healthcare-associated pneumonia (HAP), healthcare-associated urinary tract infection (HA UTI), surgical site infection (SSI), healthcare-associated Clostridium difficile infection (HA CDI), healthcare-associated neonatal sepsis and healthcare-associated primary bloodstream infection (HA primary BSI). The burden of these HAIs was measured in disability-adjusted life years (DALYs). The attributable risks for outcomes of each type of HAI were based on the results of systematic literature reviews.
For each of the six HAIs, gender and age group prevalence from the ECDC PPS was converted into incidence rates applying the Rhame and Sudderth formula. We adjusted for reduced life expectancy within the hospital population using three severity groups based on McCabe score data from the ECDC PPS.
Results: The cumulative burden of the six HAIs was estimated at 478 DALYs per 100,000 general population each year in the European Union/European Economic Area (EU/EEA). HAP and HA primary BSI represented more than 60% of the total burden with 146 and 145 DALYs per 100,000 total population respectively. HA UTI, SSI and HA CDI, and HA neonatal sepsis ranked third to sixth syndromes in terms of burden of disease. The cumulative burden of the six HAIs was higher than the total burden of all other 32 communicable diseases under surveillance that were included in the BCoDE 2015 study.
Conclusions: For the first time, we estimated the EU/EEA burden of HAIs in DALYs in 2011-2012 using a transparent and evidence based approach that allows for combining estimates of morbidity and mortality informing a comprehensive ranking suitable for prioritization and allowing for comparison with the burden of other diseases. Our results highlight the high burden of HAIs and the need for increased efforts for their prevention and control. Furthermore, our model should allow for estimations of the potential benefit of preventive measures on the burden of HAIs in the EU/EEA**.**

Presentation: Tuesday, 7 March 2017 from 11:00 - 11:30 in the Barbarossa Hall.

140/PRV
A. Clarici*[1]

No abstract has been submitted.

Presentation: Tuesday, 7 March 2017 from 11:30 - 11:50 in the Barbarossa Hall.

WORKSHOP 24
Krankheitslast durch nosokomiale Infektionen höher als erwartet - aktuelle Daten der Europäischen Prävalenzstudie (FG PR / StAG HY)
07 March 2017 • 11:00 – 12:30

143/QDV
When cleaning makes things worse: microbiome analysis reveals a dominance of potentially pathogenic bacteria in used kitchen sponges
M. Cardinale*[1], D. Kaiser[2], L. Tillmann[3], S. Schnell[1], M. Egert[2]
[1]*Justus-Liebig-University, Institute of Applied Microbiology, Giessen, Germany*
[2]*Furtwangen University, Faculty of Medical and Life Science, Institute of Precision Medicine (IPM), Microbiology and Hygiene Group, Villingen-Schwenningen, Germany*
[3]*Helmholtz Center Munich, Institute of Groundwater Ecology, Neuherberg, Germany*

In industrialized countries, the built environment (BE) is the location were people spend up to 90% of their time, rendering domestic hygiene an increasingly important research field. Many domestic habitats harbor a remarkable diversity of microorganisms, including opportunistic pathogens. In particular, kitchen environments offer ideal conditions for massive microbial growth due to high humidity and nutrient availability. Used kitchen sponges in particular are among the biggest reservoirs of bacteria of the whole house. In this study, we analyzed the bacterial microbiome of used kitchen sponges stemming from different households by means of 454–pyrosequencing of 16S rRNA genes and fluorescence *in situ* hybridization coupled with confocal laser scanning microscopy (FISH–CLSM). Pyrosequencing showed a general dominance of *Gammaproteobacteria* (especially of the family *Moraxellaceae*) and *Bacteroidetes* (family [*Weksellaceae*]). Six of the ten most abundant OTUs (defined at 97% sequence similarity) were closely related to opportunistic human pathogens. Alpha–diversity showed significant differences between individual sponges (Fig. 1A); interestingly, the microbiome structure was affected by regular sanitation of the sponges (either microwave heating or incubation in hot, soapy water, as indicated by their users): four dominant OTUs showed significantly greater proportions in sponges that were sanitized, including the risk group 2 bacteria *Chryseobacterium hominis* and *Moraxella osloensis* (Fig. 1B). Correlation of OTU occurrence patterns showed recurrent associations of potentially dangerous bacterial species. FISH–CLSM showed bacteria ubiquitously colonizing the sponge tissue, concentrating in internal cavities and on surfaces, where biofilm–like structures occurred with a density of up to 5.4*10^10 cells per

cm^3 (Fig. 2 A–D). Quantitative correlation microscopy suggested that both, less frequently replaced and more intensively used sponges have a higher bacterial load, while regular sponge sanitation just reduced bacterial abundance (but not significantly). FISH with group-specific probes confirmed the dominance of *Gammaproteobacteria*. Our study sheds more light onto a hygienically important compartment of the BE microbiome, providing substantial information to better understand its ecology and to improve domestic hygiene measures.

Fig. 1: A) Shannon–Weaver index of the sponge microbiome, calculated on the OTUs (97%); different letters indicate significantly different means (Tukey test, $p < 0.05$). B) Relative abundance of the four OTUs significantly affected by the factor "special cleaning" (G-test, ** $p < 0.005$; *** $p < 0.001$).
Fig. 2: FISH–CLSM analysis of bacterial colonization of the sponges. A) EUB338MIX–stained bacteria; B) sponge autofluorescence; C) overlap of A–B; D) 3D model of C (bacteria= spheres, sponge tissue= iso–surface). Bars: 10µm.

Figure 1

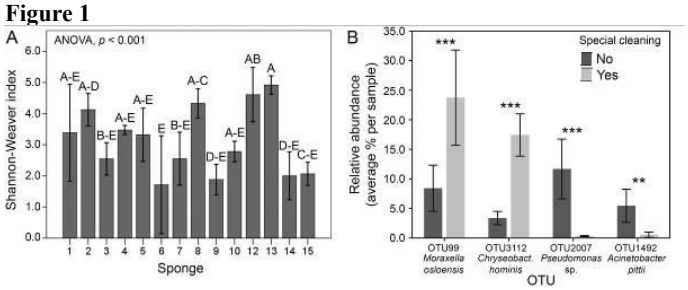
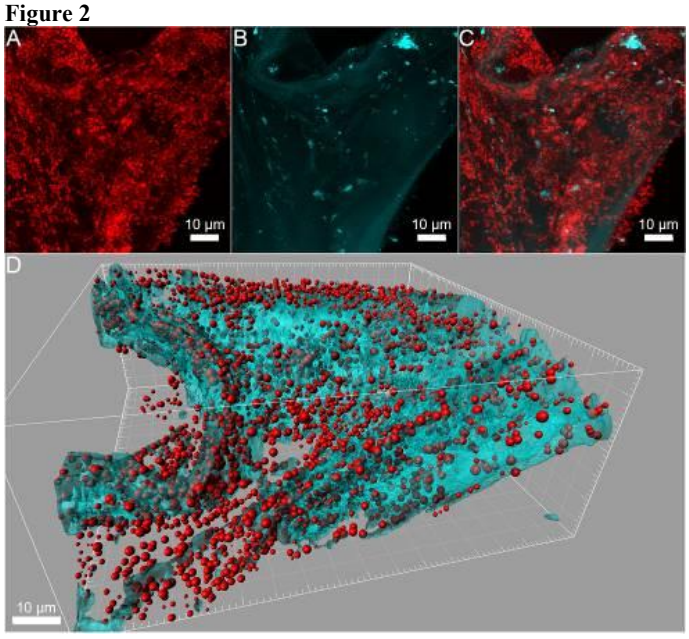


Figure 2



Presentation: Tuesday, 7 March 2017 from 11:00 - 11:15 in room 10-11.

144/DVV

Antibiotic resistance of coagulase-negative *Staphylococcus* spp. from sliced and prepacked dry- fermented hams from German retail stores

L. Kröckel*[1], S. Lick[1]
[1]*Max Rubner-Institute , Safety and Quality of Meat, Kulmbach, Germany*

The production process of sliced and prepacked dry-fermented hams allows for multiplication of staphylococci which, therefore, may be present in fairly high numbers in the final products. Usually coagulase-negative *Staphylococcus* spp. (CNS) dominate while, fortunately, *S. aureus* is less competitive in these environments. Nevertheless, antibiotic resistance is an undesirable trait also in CNS. Presently, there are no specific systematic data available for this hygienically and technologically important group of bacteria. Therefore, MIC values against 16 selected antibiotics were determined by broth microdilution for CNS isolated from hams collected from German retail stores. CNS isolates from hams of German producers were mostly identified as *S. carnosus* (48%), followed by *S. equorum* (29%) and *S. saprophyticus* (23%). In hams imported from other EU countries the dominating CNS were identified as *S. equorum* (86%), *S. carnosus* (9%) and *S. saprophyticus* (5%). In most cases the resistances were located close to the clinical breakpoints as defined by EUCAST and CLSI. No resistances were observed against kanamycin, gentamycin, mupirocin, vancomycin and, with one exception, also for rifampicin. For streptomycin the MIC value of 4 µg/ml was only exceeded by a few isolates. Against chloramphenicol 100% of the *S. carnosus* and *S. saprophyticus* isolates from German hams were resistant according to EUCAST criteria, while this was the case only for 16% and 6% of the respective isolates when CLSI criteria were applied. Oxacillin resistance was common in CNS from imported hams. Resistance against trimethoprim occurred quite frequently in CNS from both, imported and German hams. A conservative evaluation, considering only those resistances markedly above the clinical breakpoints, gave no indications for multi-resistant CNS for the most frequently isolated CNS, *S. equorum* and *S. carnosus*. Phenotypically identified resistances frequently correlated with positive PCR results for known resistance genes. Strain typing by PFGE showed that identical pulsotypes of *S. carnosus* occurred in hams from different producers. These isolates did not exhibit resistance against any of the tested antibiotics and could have been deliberately added by the manufacturers as protective cultures to control meat-born CNS and other staphylococci from the processing environments. In contrast, strain biodiversity of *S. equorum* isolates was much higher and no identical pulsotypes were observed. In conclusion, CNS isolates carrying antibiotic resistances are present at different degrees in the dominant microbiota of sliced, prepacked dry-fermented hams from German retail. Resistances, especially multiple ones, are usually only moderately expressed. Protective cultures devoid of resistances may help to constitute a hygienically more acceptable CNS microbiota on these products.

Presentation: Tuesday, 7 March 2017 from 11:15 - 11:30 in room 10-11.

145/DVV

Fifty shades of MALDI – how far can this technology go?

M. Cordovana*[1], M. Kostrzewa[2], K. Sparbier[2], M. Peer[2], M. Timke[2], M. Bienia[3], M. P. Landini[1], A. B. Pranada[3]
[1]*University Hospital Sant'Orsola-Malpighi of Bologna, Microbiology and Virology, Bologna, Italy*
[2]*Bruker Daltonik GmbH, Bremen, Germany*
[3]*MVZ Dr. Eberhard & Partner Dortmund, Department of Medical Microbiology, Dortmund, Germany*

Question: MALDI-TOF MS completely revolutionized bacteriology. But this revolution might go on as its latest applications allow further novel approaches to bacterial diagnostics. Here, we present the results of studies performed in two routine laboratories exploring MALDI-TOF MS applications to different aspects of bacteriology, regarding both species identification and detection of antibiotic resistance.
Methods: ***Bacterial identification***
Identification results (n=938,123) and time to final report for samples with anaerobes (n=5465) were evaluated before and after introduction of MALDI-TOF MS.
Positive blood cultures underwent direct bacterial identification from the pellet (n=110)or Sepsityper (n=1873).
Antibiotic-resistance detection

Spectra of 211 meticillin-resistant *S. aureus* strains were analyzed to detect PSM-*mec* peak (present in *agr*-positive MRSA strains).
Spectra of 364 *B. fragilis* were subtyped for *cfiA*-positivity. 16 *cfiA*+ strains were tested with MBT STAR-BL hydrolysis assay to verify the carbapenemase activity and to classify the enzyme as metallo-beta- lactamase.
118 carbapenem-resistant enterobacteria were tested with MBT STAR-BL hydrolysis assay (imipenem),including strains previously characterized as carbapenemase-producers by PCR (n=78), and strains resulted negative for carbapenemase-production (n=40).
Results: ***Bacterial identification***
During 2009, 285 species from 112 genera were finally reported. After introduction of MALDI-TOF MS, this progressively increased to 581 species (+104 %) from 174 genera (+55 %) in 2015.
The average time to final report decreased from about 3 days and 1 hour to about 2 days and 18 hours, and in median from 3 to 2 days.
Among positive blood cultures, with the direct (in-house)method, 66.4 % of thesamples were identified at species level, another 14.5 % at genus level. With Sepsityper method, 83.9% of the samples were identified (76.9 % at high confidence level, 7% at low confidence level).
Antibiotic-resistance detection
49/211 (23.2%) meticillin-resistant *S. aureus* strains presented the PSM-*mec* related peak, thereby could be directly classified as MRSA during standard identification .
*cfi*A-positive mass spectral pattern was detected in 32 *B. fragilis* strains (8.8%). All 16 *cfiA*+ strains tested with MBT STAR-BL hydrolyzed carbapenems, with a rate well correlated to MIC values, and were characterized as MBL.
MBT STAR-BL assay detected 76/78 known carbapenemase-producing enterobacteria. It resulted negative for all confirmed AmpC/ ESBL harbouring strains (n=20), but detected 9 carbapenem-hydrolyzing strains among strains negative to all routine methods (n=20).
Conclusions: Our findings show that MALDI-TOF MS is a very fast and efficient bacterial identification method, but also proved to be a reliable and versatile tool for the detection of different kind of antibiotic resistances.

Presentation: Tuesday, 7 March 2017 from 11:30 - 11:45 in room 10-11.

146/DVV
Data Acquisition Driven Improvement of Mycobacteria Identification by MALDI Biotyper
A. B. Pranada*[1], M. Kostrzewa[2], M. Timke[2]
[1]*MVZ Dr. Eberhard & Partner, Department of Medical Microbiology, Dortmund, Germany*
[2]*Bruker Daltonik GmbH, Bremen, Germany*

Introduction: *Mycobacterium* species are more challenging for MALDI-TOF MS analysis than other bacteria. Therefore a special sample preparation method has been developed. Nevertheless, a minor proportion of mycobacterium samples may still not be identified at all, *e.g.* because of a low amount of biomass available. Next to sample preparation, another basic approach to increase identification success of mycobacteria might be to adapt quality acceptance criteria for mass spectra.
Objectives: Some *Mycobacterium* spp. mass spectra are rejected from analysis during measurement due to missed quality criteria. Although these criteria fit well for most bacteria they may be too stringent for genus *Mycobacterium*. The aim was to adjust acquisition software quality criteria for mycobacterial mass spectra to improve proportion of accepted mass spectra and consequently of identified samples. On the other hand, species identification had to stay of constantly high specificity.
Methods: *Mycobacterium* spp. samples were processed according to MycoEX method (Bruker Daltonik). Extracts were diluted to enforce low intensity mass spectra. Mass spectra were recorded in a MALDI Biotyper system. Standard parameter settings and several test settings were applied. Different parameters as well as combinations were varied. Mass spectra were compared to Mycobacteria Library 4.0 using MALDI Biotyper Compass software (Bruker Daltonik, Germany).
In addition, 95 *Mycobacterium* spp. samples from clinical routine were analyzed with the standard acquisition method (MBT_AutoX) and with the final, optimized method (MBT_AutoX_Myco).
Results: Internal tests with diluted sample extracts revealed that quality parameters can influence each other. Therefore, also combinations of varying settings were tested. Best results without any false-positive identifications were achieved by changing signal to noise and minimal intensity threshold values.
This new mass spectra acquisition method was verfied in a clinical routine lab with 95 samples. A speculative potential side effect of lower quality mass spectra would have been lower log(score) values of still correct identification results. No such effect has been detected, average log(score) value of 95 samples was 2.2 for both the MBT_AutoX and MBT_AutoX_Myco method. Log(score) values ≥ 1.8 for mycobacteria samples did not reveal any wrong identification and state high confidence identification results.
Conclusion: Optional parameters to obtain mass spectra with sufficient information for reliable species assignment were established and implemented into the MALDI-TOF acquisition software. Poor quality mass spectra which might lead to false species identifications are still rejected from analysis. Thereby, increased sensitivity in mycobacteria identification, in particular from liquid cultures, can be obtained without loss of specificity.

Presentation: Tuesday, 7 March 2017 from 11:45 - 12:00 in room 10-11.

147/DVV
Molecular diagnosis of polymicrobial brain abscesses with Next Generation Sequencing
A. Stebner*[1], W. Geissdörfer[1], A. Ensser[2], R. Lang[1]
[1]*Universitätsklinikum Erlangen, Mikrobiologisches Institut, Erlangen, Germany*
[2]*Universitätsklinikum Erlangen, Virologisches Institut, Erlangen, Germany*

Background: Brain abscess still has a high mortality even if treated with antibiotics and neurosurgical intervention. Identification of bacteria present in the brain abscess is necessary for a targeted antibiotics therapy. Standard microbiological diagnostics of brain abscess often remains negative because of antibiotic treatment. PCR amplification of 16S bacterial RNA genes followed by sequencing is hampered by the often polymicrobial nature of brain abscesses. Next generation sequencing (NGS) is suitable for metagenomics analysis of mixed bacterial samples and has been applied successfully in different infections.
Methods: During the years 2010-2016 samples from patients with brain abscess or meningitis were collected. In addition to standard microbiological staining and culture, DNA was prepared and subjected to 16S RNA PCR. Amplicons were analyzed with the Illumina MiSeq system, the obtained sequences were blasted versus the NCBI 16S bacterial database, and the bacterial metagenomes visualized using MEGAN software. The results were compared to the results at that time from gram staining, culture and Sanger-Sequencing.
Results: In many brain abscess samples, MiSeq NGS revealed the presence of bacteria not detected by culture or conventional sequencing. These were often anaerobic bacteria, e.g. Fusobacterium nucleatum, Parvimonas micra or Porphyromonas

gingivalis, as part of a polymicrobial infection. In contrast, samples from patients with meningitis MiSeq NGS detected the single bacterial species identified also by culture or Sanger sequencing of 16S RNA amplicons.
Conclusion: These results confirm and extend earlier studies showing that next generation sequencing methods expand the spectrum of bacterial species detected in brain abscesses.

Presentation: Tuesday, 7 March 2017 from 12:00 - 12:15 in room 10-11.

148/DVV
Total Lab Automation – Introduction of urines into the automated workflow – a 6 months experience
I. Burckhardt*[1], S. Klein[1], S. Zimmermann[1]
[1]*Department for infectious Diseases, Medical Microbiology and Hygiene, Heidelberg, Germany*

Introduction: Microbiology as we know it today is dominated by manual work. This includes labeling, inoculation and transport of media. For some years now solutions for partial automation exist. However, total lab automation using one machine for labeling, inoculation, transport, incubation, reading, work-up and disposal of plates is a new development. At the Department for Infectious Diseases, University Hospital Heidelberg, we have been using a BD Kiestra TLA for processing routine patient samples since January 2016.
Aims: In July 2016 we added urines to the materials we routinely process with the TLA. This report sums up the steps of implementation and work-flow streamlining. It includes a comparison of bacteria found in urines before and after switch to automatic processing.
Materials and Methods: Initial determination of incubation and imaging programs was done using growth curves with selected ATCC strains as well as patient isolates. A special focus was set on the detection of Candida spp. because of their slow growth. Imaging conditions for the plates used were adjusted to optimized visibility of bacteria. Streamlining of workflow and optimization of imaging time points was done by re-evaluating multiply imaged plates for the visibility of growth of slow growing species.
After 4 months of routine operation we compared the number and type of bacterial species found during routine work-flow.
Results:

- The results from the growth curves allowed for a total incubation time of only 24h.
- Imaging is done after 18h and 24h.
- Finding good imaging conditions for blood agar plates is difficult and cumbersome.
- Finding good imaging conditions for chromogenic media is comparatively easy.
- We see a striking increase in the detection rate of e.g. Aerococcus urinae, Corynebacterium spp., Actinobaculum schaalii and Gardnerella vaginalis.

Summary: Transferring a classic manual workflow to a fully automated system is a demanding process. Especially for reduction of incubation times one has to have evidence that the reduced time is still long enough for detection of all species looked for. We were surprised by the fact that after we implemented the automated workflow (with a drastically reduced incubation time compared to the classic workflow) we did not find less but much more of certain bacterial species.

Presentation: Tuesday, 7 March 2017 from 12:15 - 12:30 in room 10-11.

PLENARY 05
Single-cell Genomics and Gene Expression07 March 2017 • 14:30 – 16:00

149/INV
Exploring microbial dark matter in sponge symbioses
U. Hentschel Humeida*[1]
[1] *GEOMAR Helmholtz-Zentrum für Ozeanforschung Kiel*

Single-cell genomics has advanced the field of microbiology from the analysis of microbial metagenomes where information is literally "drowning in a sea of sequences", to recognizing each microbial cell as a separate and unique entity. Single-cell genomics employs Phi29 polymerase mediated whole genome amplification to yield microgram-range genomic DNA from single microbial cells. Single-cell genomics is particularly valuable when dealing with uncultivated microorganisms, as it is still the case for many bacterial symbionts. This presentation will explore the power of single-cell genomics and (meta)-omics for sponge symbioses. Sponges (phylum Porifera) are known to host dense and diverse microbial consortia within their mesohyl matrix. Up to 47 bacterial and archaeal phyla as well as several candidate phyla representing hundreds to thousands of symbiont lineages per sponge individual have been recorded. This diverse array of microbial communities has received considerable research attention, yet much remains unknown about the mechanisms of interactions with the sponge host and their in situ ecological function(s). The talk will focus on recent insights into the genomic adaptations of sponge symbionts with a particular emphasis on metabolism and defense systems.

Presentation: Tuesday, 7 March 2017 from 14:30 - 15:00 in the Franconia Hall.

150/INV
A chemical biological approach to infection
D. Hung*[1]
[1]*Broad Institute of MIT & Harvard, Cambridge, Massachusetts, United States*

In this current era of increasing antibiotic resistance, alternative approaches to therapeutically intervening on infection are crucial. Further, even in the setting of infection that is susceptible to the current antibiotic armamentarium, significant morbidity and mortality exists, suggesting that microbiological cure alone may not necessary equate to patient cure. This talk will discuss alternative approaches to intervening on infection that are being considered and explored.

Presentation: Tuesday, 7 March 2017 from 15:00 - 15:30 in the Franconia Hall.

151/INV
***Salmonella* forms intracellular persisters with TacT**
S. Helaine*[1]
[1]*Imperial College London, MRC Centre for Molecular Bacteriology and Infection, London, United Kingdom*

Persister bacteria are non-growing, antibiotic insensitive cells, the progeny of which are sensitive to antibiotics. Bacterial persistence is a common phenotype expressed by a large number of bacterial species and is thought to be responsible for relapsing infections. During *Salmonella* infection of macrophages an important proportion of bacteria enter a persister state via the action of class II toxin-antitoxin modules. These toxin-antitoxin modules encode a stable toxin that inhibits a vital cellular process and a labile, neutralising antitoxin, which is degraded under conditions of stress but otherwise binds and inactivates the toxin. We investigate the activity of three of these toxins, which are

acetyltransferases and how bacteria recover from the persistent state.

Presentation: Tuesday, 7 March 2017 from 15:30 - 16:00 in the Franconia Hall.

PLENARY 06
Host Niches and Infection Models
07 March 2017 • 14:30 – 16:00

152/INV
Impact of phages on *Vibrio cholerae* infection and their use in preventing cholera
A. Camilli*[1], M. Yen[1], L. Cairns[1], A. Wong[1]
[1]*Tufts University, School of Medicine, Department of Molecular Biology & Microbiology and Howard Hughes Medical Institute, Boston, Massachusetts, United States*

Bacteriophages (phages) play major roles in the ecology and evolution of essentially all bacteria including pathogenic species. By studying hundreds of cholera patient stool samples from clinics in Bangladesh and Haiti we found that most cholera patients shed high titers of at least one of three distinct species of *V. cholerae*-specific virulent (lytic) phage. We provide evidence that these phages prey extensively on *V. cholerae* within the human gastrointestinal tract, thus impacting the infection, dissemination, transmission and evolution of *V. cholerae*. Using genetic and phenotypic analyses we have begun to reveal the biology of these phages and important details of their ongoing arms race with *V. cholerae*. We have shown that the receptors for these phages are important virulence factors, and thus most phage-resistant escape mutants, which arise from mutations in the genes required for the production of these receptors, are avirulent. By combining these phages we have developed a cocktail that can prevent cholera when administered to animals up to 24 hours prior to challenge. The combination of phages prevents the appearance of mutants that can escape predation by all three phages. Consistent with these phages being specific for *V. cholerae*, we have shown that administering the phage cocktail to healthly animals does not disrupt the normal gut microbiota.

Presentation: Tuesday, 7 March 2017 from 14:30 - 15:00 in the Barbarossa Hall.

153/INV
Genome-wide detection of virulence and host recognition determinants in *Pseudomonas aeruginosa*
S. Lory*[1]
[1]*Harvard Medical School, Department of Microbiology and Immunobiology, Boston, Massachusetts, United States*

Modern approaches of molecular biology, particularly exploitation of next generation sequencing technologies have provided an opportunity to study the complex interaction of bacterial pathogens with their hosts. Results of a series of experiments, using transposon site mapping (TnSeq) in studying the fitness of individual mutants of *Pseudomonas aeruginosa* within a pool of mutagenized cells, in several infection models, will be discussed. These studies of fitness and host adaptation uncovered novel virulence mechanisms as well as provided new insights into the role of innate host defenses in controlling infections. This work also generated new insights into the diverse role of small regulatory RNAs, controlling host adaptation and stress survival functions. Implications of findings generated by new tools of functional genomics in defining the roles of uncharacterized genes and their products will be also discussed.

Presentation: Tuesday, 7 March 2017 from 15:00 - 15:30 in the Barbarossa Hall.

154/INV
Infection, inflammation and cancer in the stomach - organoids as new model
S. Bartfeld*[1]
[1]*Research Center for Infectious Diseases, Wuerzburg, Germany*

The epithelial lining of the gastrointestinal tract acts as physical and immunological barrier between the microbes of the gut and the body. Our group is interested in host-microbe interactions at this barrier, particularly in the stomach. Here, the first line of defense, the innate immune defense of the epithelium determines the outcome of an encounter with a pathogen. The bacterium *Helicobacter pylori* evades eradication by the host's defense mechanisms and establishes chronic infection, which can ultimately lead to gastric cancer. To study host-pathogen interaction, we use a relatively new model system of primary human and mouse cultures of epithelial cells, called organoids. This culture model is based on recent advances in stem cell research and comprises of a 3-dimensional (3D) multicellular primary cell culture that closely mimic the in vivo organization of different cell lineages, thus resembling mini versions of the original organs. Inflammatory responses are organ-specific and infection of gastric organoids with *Helicobacter pylori* reveals even cell type-specific inflammatory responses. Organoids grown from tumors as well matched healthy tissue allow evaluation of drug responses, thus indicating that organoids not only faithfully represent the healthy epithelium in vivo, but also recapitulate disease and are a useful model for the study of infection and the development of new drugs.

Presentation: Tuesday, 7 March 2017 from 15:30 - 16:00 in the Barbarossa Hall.

WORKSHOP 25
Molecular and infection epidemiology (FG MS / StAG RK)
07 March 2017 • 18:00 – 19:30

159/RKV
Two outbreaks of Legionnaires Disease in the hanseatic city of Bremen- One epidemic strain- A microbiological investigation
A. Gründel*[1], M. Petzold[1], M. Lelgemann[2], M. Berges[3], K. Lück[1], C. Lück[1]
[1]*Institut für Medizinische Mikrobiologie/ TUD, Dresden, Germany*
[2]*Gesundheitsamt, Bremen, Germany*
[3]*Landesuntersuchungsamt , Bremen, Germany*

Objectives: Two outbreaks of Legionnaires" Disease (LD) were reported in the city of Bremen. The first wave of LD cases was reported in November/December 2015 followed by a second wave in February/March 2016. Isolates could be obtained from patient samples and were analyzed by using monoclonal antibodies (mAb) and sequence based typing (SBT). In addition, several environmental samples were analyzed in order to identify the potential source.
Methods: LD cases were confirmed by *L. pneumophila* PCR (n=20), *L. pneumophila* SG 1 PCR (n=5), urinary antigen test (n=42) or culture (n=13) in the laboratory. Working and living places of the patients were mapped to narrow the area of potential environmental sources. Several cooling towers of seven companies were sampled. In total 549 *L. pneumophila* Sg1 isolates were identified and further typed by a panel of six mAb´s (mAb 81-2, mAb 48-3, mAb 8-4, mAb 3, mAb 20-1, mAb 30-1) using an automated ELISA.

Results: 19 cases of LD with one fatal were reported during the first wave of outbreak. During the second outbreak 26 LD notifications were reported with another fatal. In both outbreaks, the epidemic strain was characterized as *L. pneumophila* serogroup (Sg1), mAb-subgroup Benidorm, and the novel sequence type (ST) 2151. Several putative sources were sampled, however, the epidemic strain could not be isolated.
Conclusion: The outbreaks in Bremen proved to be in fact one repeated outbreak with biphasic character having putatively the same source of infection. Despite the high number of *L. pneumophila* isolates obtained from different environmental samples, we were not able to detect the outbreak strain so far. In total, 43 LD cases and two deaths were reported. The case-fatality rate was 4.65 %. The lack of a central registry for CT"s in Germany made the search for potential sources difficult. However, the automated ELISA helped to subgroup rapidly and reliable the high amount of samples.

Presentation: Tuesday, 7 March 2017 from 18:00 - 18:15 in room 13.

160/RKV
Invasive *Neisseria meningitidis* and *H. influenzae* isolates submitted to the National Reference Laboratory for Meningococci and *H. influenzae* (NRZMHi) 2016
T. T. Lam*[1], H. Claus[1], U. Vogel[1]
[1]*Wuerzburg University, Institute for Hygiene and Microbiology, Wuerzburg, Germany*

Introduction: The NRZMHi carries out laboratory surveillance of invasive meningococci and *H. influenzae* in Germany. Its tasks include species confirmation, typing, and monitoring of antibiotic resistance. Voluntary submissions are yearly matched with data from the Robert Koch Institute to consolidate the quality of surveillance in Germany.
Aims: Analysis of invasive meningococcal and *H. influenzae* isolates in Germany 2016 to provide descriptive epidemiologic data.
Materials and Methods: Isolates were analysed by phenotypical and molecular methods. *N. meningitidis* was typed by slide agglutination and genetic analysis of capsule genes. Additional finetyping was done by *por*A and *fet*A sequencing, and MLST. *H. influenzae* serotyping was carried out by slide agglutination and PCR for *bex*A. Antibiotic resistance was tested by E-Test according to EUCAST. Penicillin-resistant *N. meningitidis* were analysed by *pen*A sequencing. Phenotypic β-lactamase-negative ampicillin resistant (BLNAR) or imipenem resistant *H. influenzae* were analysed by *fts*I sequencing.
Results: Coverage of the surveillance data, serotype distribution and antibiotic resistance will be presented for 2016. As of Nov 24th, the NRZMHi has analysed 828 samples. According to our preliminary data, invasive meningococcal disease was diagnosed in 257 patients; 227 vital invasive isolates were analysed. The most common serogroup was B (n=150; 59%), followed by C (n=54; 21%). Serogroups W (n=25; 10%) and Y (n=23; 9%) were less frequent. Compared to previous years, serogroup W was significantly more prevalent. Reduced susceptibility to penicillin was found in 41% (n=90), mostly due to intermediate susceptibility; 16 isolates (7%) were resistant. All isolates were cefotaxim susceptible.
Invasive *H. influenzae* infection was diagnosed in 399 patients. The majority were people of age (50+ years: n=320; 80%), only 11% (n=43) were aged < 15 years. Infections were mostly due to unencapsulated strains (NTHi: n=322; 81%). The most common capsule type was f (Hif: n=55; 14%), serotype b (Hib) was rare (n=11; 3%). Antibiotic resistance data available for 385 isolates showed ampicillin resistance in 18% (n=71) with 6% BLNAR (n=25). Imipenem resistance testing in invasive *H. influenzae* had been started in 2016 and was surprisingly high (n=66; 17%). All isolates were susceptible to cefotaxim and meropenem.
Conclusions: *H. influenzae* infections have outnumbered meningococcal disease. This is a result of a steady decline of *N. meningitidis* infections caused by serogroup B, but also C, against which vaccination is recommended. Moreover, incidence of invasive NTHi infections has been rising markedly among elderly patients. Resistance of both species against first line antibiotics was not critical. However, reduced susceptibility to penicillins should be surveyed. Further investigation on the imipenem resistance of *H. influenzae* is needed.

Presentation: Tuesday, 7 March 2017 from 18:15 - 18:30 in room 13.

161/RKV
Development of a high-throughput screening assay for the detection of anti-*Bartonella henselae* IgG antibodies
D. Villinger*[1], W. Ballhorn[1], H. Podlich[1], A. Hillebrecht[1], Y. Regier[1], S. Besier[1], V. A. J. Kempf[1]
[1]*University Hospital, Goethe-University, Institute for Medical Microbiology and Infection Control, Frankfurt a. M., Germany*

Bartonella henselae is a zoonotic pathogen causing cat scratch disease (CSD) and other infections. Despite its global occurrence, epidemiological data are rather sparse or only represent small sample numbers.
Indirect immunofluorescence assays (IFA) are well established tests for the diagnosis of *B. henselae* infections. Since these tests are immunofluorescence microscopy-slide based, handling is inconvenient when processing large sample numbers. We designed a high-throughput screening assay for the detection of *B. henselae* IgG antibodies which allows the generation of seroepidemiological data of *B. henselae* infections on a larger scale.
For antigen preparation, HeLa-229 cells were infected with *B. henselae* (strain Houston-1) in a 96-well plate format. A three-day processing protocol was designed: day 1 antigen preparation; day 2 immunofluorescence assay; day 3 microscopic evaluation. A total amount of 5.215 sera was processed. All sera were assayed at a breakpoint titer of 1:320 (according to CDC-guidelines). To check for inter-assay specificity, 238 randomly picked sera were counterchecked with the commercially available immunofluorescence tests from routine diagnostics (Euroimmun, Lübeck). To evaluate the assay specificity for *B. henselae* IgG antibodies and to exclude cross-reactivity, sera with high antibody titers against other pathogens (e.g. *Anaplasma phagocytophilum, Brucella spp., Coxiella burnetii, Epstein-Barr-virus, Leptospira interrogans, Mycoplasma pneumoniae*, *Treponema pallidum, Rickettsia rickettsii and Rickettsia typhi*) were used. Furthermore, reactivity was also assessed using various other *Bartonella* strains (*B. henselae* Marseille, *B. henselae* Marseille BadA-, *B. quintana*).
The assay turned out to be (i) easy to handle and (ii) reliable (congruency to standard laboratory methods: ~84%) although the reasons for non-congruency are unclear. Therefore, the newly developed high-throughput IFA is a step forward to analyze large amounts of human serum samples and to collect, e.g. seroepidemiological data.

Presentation: Tuesday, 7 March 2017 from 18:30 - 18:45 in room 13.

162/MSV

Signature patterns of SNP in genomes of *Mycobacterium tuberculosis* for identification of clades and drug resistance variants

O. Reva*[1]
[1]*University of Pretoria, Biochemistry, Pretoria, South Africa*

Multidrug resistant infections is a scourge of humanity casting us back to the pre-antibiotic era. According to WHO, an estimated 480,000 people developed multidrug-resistant tuberculosis (MDR-TB) annually around the globe. Timely diagnostics of MDR-TB is important for selecting an appropriate regiment of treatment. This work introduces a computational approach of analysis of genomic polymorphisms for identification of Mtb clades and drug resistant variants by using NGS sequencing data. Analysis of complete genome sequences of 1,623 strains of Mtb from http://mtb.dobzhanskycenter.org/ revealed patterns of clade and lineage specific SNP including drug resistance mutations. A database of pairs of SNP showing statistically reliable positive and negative linkage disequilibrium dependences was created. Non-randomly distributed SNP then were grouped by using Markov clustering into diagnostic patterns of SNP suitable for identification of clades, sub-clades and lineages of Mtb by sequence data in numerous file formats including raw fastq files of NGS reads. Clade identification program was implemented as a Web-service at http://mtbclade.bi.up.ac.za/. Analysis of distribution of known drug resistance mutation showed that each of them was associated in average with a pattern of 50 other SNP comprising compensatory mutations and neutral signature polymorphisms that allowed a robust identification of drug resistant variants by sequence data. The program differs from other similar tools of Mtb clade identification such as PhyTB by allowing users to submit initial data files in different formats. Moreover, study of functions of the genes bearing clade and lineage specific mutations can shed light on intrinsic processes and driving forces of the evolution of Mtb, and also to aid in identification of possible targets for new anti-tuberculosis drugs.

Presentation: Tuesday, 7 March 2017 from 18:45 - 19:00 in room 13.

163/MSV

Rates of spontaneous mutation, short-term evolution, and *in-vivo* growth of pathogenic bacteria

A. K. Szafranska*[1,2], V. Junker [1], U. Nübel [1,2]
[1]*Leibniz Institute DSMZ, Braunschweig, Germany*
[2]*German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig , Braunschweig, Germany*

Introduction: Recently, population genomic analyses have provided estimates of short-term evolutionary rates for a number of human pathogenic bacteria. Annual rates of base substitution differ markedly between different species of bacteria, for as yet unknown reasons. One parameter that is fundamentally important for understanding the dynamics of bacterial evolution is the initial, spontaneous mutation rate, hence the probability of a mutation to occur in each generation.
Objectives: We determined spontaneous mutation rates for diverse strains of *Staphylococcus aureus*, *Clostridium difficile*, and multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae*. We compared this data to in-vitro mutation rates reported in the literature, and related these mutation rates per generation to annual base substitution rates.
Materials & Methods: We performed classical fluctuation analyses. Mutation rates were calculated employing the maximum likelihood method using and pairwise comparisons of mutation rates were assessed using likelihood ratio tests.
Results: Spontaneous mutation rates were largely uniform among clinical isolates within each bacterial species, but we noted significant differences between species. Comparisons of mutation rates to short-term base substitution rates suggested striking differences of average *in-vivo* growth rates among different pathogenic bacteria.
Conclusion: Our results suggest that diverse bacterial pathogens experience very high average *in-vivo* growth rates that resemble maximum growth rates observed in laboratory cultures.

Presentation: Tuesday, 7 March 2017 from 19:00 - 19:15 in room 13.

164/MSV

***Staphylococcus aureus* was repeatedly introduced to the African continent**

L. Strauß*[1], S. Abdulla[2], A. Alabi[3], M. Garrine[4], A. Germann[5], V. Helms[6], T. Kazimoto[2], W. Kern[7], M. Lebughe[8], I. Mandomando[4], D. Mumba[8], J. J. Muyembe-Tamfum[8], G. Peters[9], P. Phaku[8], U. Ruffing[10], F. Schaumburg[9], D. Vubil[4], M. Herrmann[11], A. Mellmann[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*
[2]*Ifakara Health Institute, Bagamoyo, Tanzania*
[3]*Albert-Schweitzer-Hospital, Lambaréné, Gabon*
[4]*Manhiça Health Research Centre, Manhiça, Mozambique*
[5]*Fraunhofer Institute for Biomedical Engineering, Sankt Ingbert, Germany*
[6]*Saarland University, Saarbrücken, Germany*
[7]*University Hospital Freiburg, Freiburg, Germany*
[8]*Université de Kinshasa, Institut National de Recherche Bio-Médicale, Kinshasa, Congo*
[9]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*
[10]*Saarland University, Institute of Medical Microbiology and Hygiene, Homburg/Saar, Germany*
[11]*University Hospital Münster, Münster, Germany*

Introduction: *Staphylococcus aureus* is an opportunistic bacterium that asymptomatically colonizes 25-35% of the human population. Although severe infections with *S. aureus* are common in countries of sub-Saharan Africa, little is known about the bacterium"s epidemiology and population dynamics in this particular world region.
Objectives: Our aim was to investigate the relationship between *S. aureus* isolated in Africa and *S. aureus* isolated in Germany using phylogenomics.
Materials & Methods: We performed Illumina whole genome shotgun sequencing of currently 738 community-associated *S. aureus* isolated from patients (n=375) and healthy volunteers (n=363) from Germany (n=371), as an example for a well-studied, temperate and industrialized region, and sub-Saharan Africa (Tanzania [n=130], Gabon [n=35], Democratic Republic of Congo [n=186], Mozambique [n=17]). The allelic profiles of 1,861 core genome genes (cgMLST) were used to determine the neighbour-joining (NJ) phylogeny of all isolates. Moreover, we performed *in silico* multilocus sequence typing (MLST).
Results: Our 738 isolates were assigned to 115 different MLST sequence types (ST), clustering in 39 clonal complexes (CC) according to the NJ phylogeny. Eighteen CC (e.g. CC7, CC398, CC22) exclusively comprised isolates from Germany (n=139), while eight CC (e.g. CC88, CC1292, CC80) exclusively comprised African isolates (n=38). "African" CCs were found in different phylogenetic branches. Within the CCs occurring in both regions, African isolates were usually found in monophyletic subgroups, containing no German isolates. Our results indicate that the ancestors of the African *S. aureus* included in our dataset were introduced to the continent in single independent transmission events, followed by regional spread and diversification.
Conclusion: Our results show that the population structures of African and German *S. aureus* differ. The results hint that *S. aureus* was repeatedly introduced to Africa, potentially from Europe, and might not be native to the continent. The fact that we do not find isolates from Germany in the "African subgroups" indicates a limited transmission from Africa to Germany.

We hypothesize that, if the assumption is true that the modern *Homo sapiens* evolved in Africa, the adaptation of *S. aureus* to the human host has happened after the emigration of prehistoric men from Africa. However, extended analyses using global isolates and Bayesian statistics are necessary to evaluate our hypothesis.

Presentation: Tuesday, 7 March 2017 from 19:15 - 19:30 in room 13.

WORKSHOP 26
Eukaryotic Pathogens (FG EK)
07 March 2017 • 18:00 – 20:00

165/EKV

The role of histone acetylation in the control of gene expression during *P. falciparum* gametocyte development and transmission to the mosquito.

C. Julius Ngwa*[1], M. Kiesow[1], O. Papst[1], J. Möcking[1], L. M. Altenhofen[2], M. Filarsky[3], T. Voss[3], M. Llinás[2], G. Pradel[1]
[1]*RWTH University, Division of Cellular and Applied Infection Biology, Aachen, Germany*
[2]*Penn State University, Department of Biochemistry and Molecular Biology & Center for Malaria Research, Pennsylvania, United States*
[3]*Swiss Tropical and Public Health Institute, Department of Medical Parasitology and Infection Biology, Basel, Switzerland*

Introduction: Histone acetylation is an important post-translational modification regulated by the enzymes histone acetyltransferases (HATs) and histone deacetylases (HDACs). This process is vital in the regulation of gene expression and other key processes in eukaryotes. In *P. falciparum* the agent responsible for malaria tropica, histone acetylation has been shown to control the gene expression of several genes involved in the asexual replication cycle. In the sexual stages, which are crucial for the transmission of the disease, little is known.
Objective: We here aimed to investigate the role of histone acetylation in the regulation of gene expression in *P. falciparum* gametocytes during maturation and following transmission to the mosquito.
Materials and Methods: The effect of HDACs on gene expression was carried out using a microarray-based transcriptome study on gametocytes treated with the HDAC inhibitor trichostatin (TSA). Indirect immunofluorescence assays (IFAs) and western blotting were used to detect histone acetylation and hyperacetylation in gametocytes following TSA treatment. Chromatin immunoprecipitation (Chip) analysis was also used to confirm association of some deregulated genes to histones. One deregulated gene named *Pf*RNF1 was further characterised by IFAs, western blotting and Chip.
Results: Comparative transcriptomics between untreated and inhibitor-treated gametocytes identified over 200 genes, most of which are associated with cell cycle, gene regulation and exported proteins, that were more than 2-fold deregulated. Using IFAs with anti-H3K9ac and H4K4ac antibodies, we could detect histone-acetylated proteins in gametocytes (Stages II-V) and show by Western blotting that treatment of immature and mature gametocytes with TSA results in significant hyperacetylation. We also confirmed by Chip analysis using anti-H3K9ac and H4K4ac antibodies that selected deregulated genes following TSA treatment are associated to histones. Furthermore, characterization of one of the deregulated genes encoding for the ring finger protein *Pf*RNF1 suggests a role of this potential E3 ligase in ubiquitin-mediated pathways crucial during gametocyte development.
Conclusion: These studies indicate that the control of gene expression by histone acetylation and deacetylation plays a significant role during gametocyte development and gametogenesis. HATs and HDACs in *P. falciparum* may therefore represent promising targets for transmission-blocking intervention strategies.

Presentation: Tuesday, 7 March 2017 from 18:00 - 18:15 in room 10-11.

166/EKV

Fitness costs of drug resistance in clinical *Candida albicans* isolates

C. Popp*[1], I. Hampe[1], T. Hertlein[1], K. Ohlsen[1], J. Morschhäuser[1]
[1]*Institute for Molecular Infection Biology, University of Würzburg, Germany*

Candida albicans can develop resistance to the widely used antifungal agent fluconazole, which inhibits ergosterol biosynthesis. Resistance is often caused by gain-of-function mutations in the transcription factors Mrr1, Tac1, and Upc2, which result in constitutive overexpression of multidrug efflux pumps and ergosterol biosynthesis genes, respectively. However, the deregulated gene expression that is caused by hyperactive forms of these transcription factors also reduces the fitness of the cells in the absence of the drug. In cocultivation experiments, strains containing gain-of-function mutations in Mrr1, Tac1, and/or Upc2 are outcompeted by an isogenic wild-type strain. To investigate whether fluconazole-resistant, clinical *C. albicans* have overcome the fitness costs of drug resistance, we assessed the relative fitness of *C. albicans* isolates containing resistance mutations in these transcription factors in competition with matched drug-susceptible isolates from the same patients. Most of the fluconazole-resistant isolates were outcompeted by the corresponding drug-susceptible isolates when grown in rich medium without fluconazole. On the other hand, some resistant isolates with gain-of-function mutations in *MRR1* did not exhibit reduced fitness under these conditions. Introduction of the same mutations into the endogenous *MRR1* alleles of the wild-type reference strain SC5314 resulted in reduced competitive fitness, indicating that the clinical isolates had acquired compensatory mutations that mitigated the fitness costs caused by their hyperactive Mrr1. In a mouse model of disseminated candidasis, three out of four tested fluconazole-resistant clinical isolates did not exhibit a significant fitness defect. However, all four fluconazole-resistant isolates were outcompeted by the matched susceptible isolates in a mouse model of gastrointestinal colonization. These results indicate that the fitness costs of drug resistance in *C. albicans* are not easily remediated, especially when proper control of gene expression is required for a successful adaptation to life within a mammalian host.

Presentation: Tuesday, 7 March 2017 from 18:15 - 18:30 in room 10-11.

167/EKV

Regulation of *ECE1* transcription in *Candida albicans*

R. Martin*[1], E. Garbe[1], O. Kurzai[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute, Septomics Research Center, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute, National Reference Center for Invasive Fungal Infections, Jena, Germany*

Introduction: *ECE1* has been known for several years as one of the most transcribed genes in *C. albicans* hyphae. In yeast cells however, it is barely expressed and can therefore be used as marker for filamentation-associated gene expression in this human fungal pathogen. Recently, it was shown that Ece1 protein is processed into different peptides. One of them is cytolytic and contributes to damage of host cell membranes. Therefore, the understanding of the regulation of this virulence gene can help to better understand fungal virulence.

Objectives: As other core filamentation response genes, *ECE1* has a very long upstream intergenic region of more than 3000bp. The first aim of this work was to find out which parts of this intergenic region are required for proper regulation of *ECE1* in yeast and hyphal cells. Consequently, we examined which transcription factors contribute to the regulation of this gene.
Material and Methods: At first, we used RACE PCR to identify the 5 untranslated region of *ECE1*. Parts of the intergenic region consisting of the first 500bp up to the whole length region 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. QRT PCR and the GFP reporter system were also used to study deletion mutants lacking putative regulators of *ECE1*.
Results: *C. albicans* strains where GFP was under control of the first 1000bp of the intergenic region did not show any detectable fluorescence. The minimum size of a promoter which resulted in GFP signals was 1200bp, but it was less intense than those with 1500bp or more. This was surprising as the 5UTR has only a size of 50bp. The *ECE1* promoter possesses putative binding sites for a variety of transcription factors. An *ECE1*prom-GFP-based screening of deletion mutants revealed that Ahr1 might have a crucial role in the activation of *ECE1* transcription.
Conclusions: Despite a small 5 UTR, the promoter of the *ECE1* gene is large and contains of at least 1200bp. However, high level transcription of the gene requires 1500bp and more. The transcription factor Ahr1 contributes to ECE1 expression, although the detailed mechanism is subject of further experiments.

Presentation: Tuesday, 7 March 2017 from 18:30 - 18:45 in room 10-11.

168/EKV
A hybrid transcription network controls *Candida glabrata* iron homeostasi
F. Gerwien[1], F. Hille[1], J. Linde[2], P. Kämmer[1], L. Kasper[1], B. Hube[1], S. Brunke*[1]
[1]*Hans-Knöll-Institut, Microbial Pathogenicity Mechanisms, Jena, Germany*
[2]*Hans-Knöll-Institut, Systems Biology & Bioinformatics, Jena, Germany*

Question: The regulation of iron homeostasis is a central element in the successful infection of mammals by fungi, as iron levels can change substantially during the infection process. Among the important fungal pathogens, like *Candida albicans* or *Aspergillus fumigatus*, iron uptake and consumption is regulated by homologous systems: a GATA factor inhibits iron uptake under iron replete conditions, and a CCAAT binding complex inhibits iron consumption under limitation. In *C. albicans* and related fungi, Sef1 additionally up-regulates iron uptake in times of need. *Candida glabrata*, however, is closely related to the normally benign baker's yeast, *Saccharomyces cerevisiae*, which employs an evolutionary highly derived iron homeostasis system, based on an activator of iron uptake, Aft2, and down-regulation of iron-consuming processes by mRNA degradation. We therefore asked which system is employed by *C. glabrata* – the one common to pathogenic fungi or the alternative system used by the non-pathogenic baker's yeast?
Methods: A library of deletion mutants covering a large fraction of the *C. glabrata* genome was screened under iron limitation and iron abundance for growth defects. Transcriptome analyses under the same conditions guided creation of additional mutants and allowed to follow the time course of the cellular reaction to changes in available iron.
Results: We found that in *C. glabrata* Aft1, an Aft2 ortholog, is the main factor for up-regulation of iron uptake (*FTR1*, *FET3*, *SIT1*) and iron recycling genes (*HMX1*) under iron limitation. Additionally, we found that post-transcriptional degradation of mRNAs coding for iron-requiring enzymes is mediated by a 3' consensus motif. Under iron depletion, mRNAs e.g. from *ACO1*, *CTA1* and *HEM15* are thus targeted by the RNA binding protein Cth2. While these observations resembled the *S. cerevisiae* system rather than *C. albicans*, we also found a function of *C. glabrata* Sef1 in iron-dependent regulation especially of *ACO1*, *IDH1* and *IDH2*. Importantly, both Aft1 and Sef1 contributed to survival of *C. glabrata* in a human blood infection model.
Conclusion: *C. glabrata* seems to employ an unusual hybrid regulatory system to respond to the external supply of iron. It resembles *S. cerevisiae* in its reliance upon an Aft factor and mRNA degradation for up- and down-regulation of iron acquiring and requiring processes, respectively. However, parts of *C. glabrata*'s response to iron also depend on the Sef1 activator found in other pathogenic fungi like *C. albicans*. This puts *C. glabrata* into the unique position of a human pathogen with an evolutionary intermediate iron acquisition system, combining traits from both, non-pathogenic and pathogenic fungi into a novel regulatory network.

Presentation: Tuesday, 7 March 2017 from 18:45 - 19:00 in room 10-11.

169/EKV
***Candida albicans* counteracts Dectin-mediated antifungal host response**
N. Reiher*[1,2], M. J. Reza[1], P. F. Zipfel[1,3]
[1]*HKI Leibniz Institute for Natural Product Research and Infection Biology, Infectionbiology, Jena, Germany*
[2]*CSCC Center for Sepsis Control and Care, Jena, Germany*
[3]*Friedrich-Schiller-University, Jena, Germany*

Introduction: Detection of foreign invaders is the first step to induce an effective immune response. Therefore host immune cells express a broad range of pattern-recognition-receptors (PRRs). These PRRs bind highly conserved microbial-associated-molecular-patterns (MAMPs), which are shared by infectious microbes. Dendritic cells (DCs), which link innate to adaptive immunity, express the PRRs Dectin-1 and Dectin-2. After binding to MAMPS the PRRs trigger specific signaling pathways which lead to the production of different cytokines that influence the inflammatory response. Dectin-1 and Dectin-2 are responsible for the recognition of the fungal cells wall proteins β-glucan and mannan epressed by candida yeast and hyphae. *Candida albicans* is a dimorphic pathogenic fungus that colonizes skin and mucosal surfaces in humans and resides mostly as a harmless commensal. However, in immunosuppressed individuals candida can induce severe systemic infections, which can end up in sepsis with deadly outcome. Mortality and morbidity rates of patients with disseminated candida infections are still unacceptable high, whereas the development of antifungal therapies is so far only partially successful. For this reason it is important to investigate the interaction of *C. albicans* and human innate immune cells. In order to understand the molecular mechanism of fungal counterstrike and immune interference.
Objective: Aim of this project is to characterize how *C. albicans* by secreting the immune evasion protein CRASP11 evades Dectin-1 and Dectin-2 recognition by human dendritic cells. We are defining general features of the fungal counterstrike to Dectin-mediated pattern recognition. We also aim for the characterization how candida CRASP11 influences DC function and cytokine response.
Methods: Protein interactions, Biolayer Interferometry, Confocal Microscopy, Cytokine ELISA, Flow Cytometry, Immunoprecipitation, Western Blot, immunological analysis
Results: We have identified a new candida protein, i.e. CRASP11, which binds to Dectin-1 and Dectin-2 on human dendritic cells and modulates DC function. Soluble CRASP11 upon binding to both Dectin receptors induces the production of anti-inflammatory IL-10 and blocks the secretion of pro-inflammatory cytokines TNF-α and IFN-γ. In addition CRASP11 blocks LPS induced activation and maturation of dendritic cells

and down regulates expression of the maturation markers CD80, CD83 and CD86 to keep the DCs in an immature state.
Conclusion: *C. albicans* escapes dectin-mediated pattern recognition by secretion of CRASP11. The immune evasion protein CRASP11 binds to both Dectin-1 and Dectin-2 and modulates the inflammatory response of human dendritic cells. CRASP11 induces anti-inflammatory IL-10 and simultaneously down-regulates the expression of pro-inflammatory cytokines. Thereby CRASP11 generates an immunosuppressive phenotype in DCs.

Presentation: Tuesday, 7 March 2017 from 19:00 - 19:15 in room 10-11.

170/EKV
A novel killing assay uncovers a role of *Aspergillus fumigatus* cell wall integrity signaling to resist human neutrophil granulocytes.
D. Ruf[1], V. Brantl[1], V. Loiko[1], K. Dichtl[1], J. Wagener*[1]
[1]*Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Ludwig-Maximilians-Universität München, München, Germany*

In healthy humans, neutrophil granulocytes play a key role in the defense against fungal infections caused by the mold *Aspergillus fumigatus*. We recently established a novel assay to quantify killing of *Aspergillus* hyphae by neutrophil granulocytes. The assay relies on microscopic evaluation of the viability of single *Aspergillus* hyphae. In our present study we used this assay to screen *A. fumigatus* mutants impaired in cell wall integrity maintenance for increased susceptibility to human neutrophils. We show that survival of *A. fumigatus* hyphae exposed to neutrophils relies on specific components of the cell wall integrity signaling pathway. In addition, we identified a Rho GTPase whose overexpression significantly reduces the susceptibility of *A. fumigatus* to killing by neutrophil granulocytes. Interestingly, this GTPase also affects survival of *A. fumigatus* exposed to echinocandin antifungals. This suggests a more general role of the Rho GTPase in stress resistance of the fungus. Our results underline the suitability of our novel killing assay to detect discrete differences in the susceptibility of *A. fumigatus* mutants and demonstrate the importance of cell wall integrity maintenance to withstand killing by neutrophil granulocytes.

Presentation: Tuesday, 7 March 2017 from 19:15 - 19:30 in room 10-11.

171/EKV
***Candida albicans* and *Proteus mirabilis* interactions enhance epithelial cell damage**
J. Niemiec[1,2], M. Kapitan[1,2], I. Jacobsen*[2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Microbial Immunology, Jena, Germany*
[2]*Center for Sepsis Control and Care, Jena, Germany*

The yeast *Candida albicans* colonizes the gastro-intestinal tract of most humans asymptomatically. However, if the local equilibrium of host, fungus and bacterial microbiota is disturbed, *C. albicans* can translocate from the gut into deeper tissue and cause life-threatening infections. Bacterial-fungal interactions are likely to occur throughout both processes and possibly influence disease risk and outcome.
We therefore analysed interactions between sepsis-relevant intestinal bacteria and *C. albicans* in co-cultures and coinfection using an *in vitro* models, such as epithelia composed of HT29-MTX and C2BBe1 cells. All experiments were performed under normoxia (21% oxygen) and controlled low oxygen (1%) , the later to mimic conditions in the gut.
We found that coinfection of enterocytes with *C. albicans* and several wild type isolates of the gram-negative bacterium *Proteus mirabilis* led to significantly enhanced cell damage both under normoxic and low oxygen conditions. This enhancement was depended on the *P. mirabilis hpmA* gene, encoding a hemolysin, and was only moderately affected by *P. mirabilis* swarming motility. Synergistic interactions between fungus and bacteria were also observed in the absence of host cells: *C. albicans* supernatants promoted *P. mirabilis* growth. Bacteria were found to attach to *C. albicans* hyphae on agar under low oxygen and in normoxic liquid culture without affecting *C. albicans* filamentation. However, *P. mirabilis* also inhibited fungal growth in liquid media, indicating that the interactions between these two species are not solely synergistic. In contrast to epithelial damage, inhibition of *C. albicans* growth was independent of *P. mirabilis* hemolysin.
To further characterize the interactions between *C. albicans* and *P. mirabilis*, we are currently performing scanning electron microscopy of co-cultures and coinfections and test fungal mutants to identify *C. albicans* virulence factors that contribute to synergistic damage.
To date, our data indicate vivid interactions between *C. albicans* and *P. mirabilis* – two common colonizers of the human gut. Our findings contribute not only to the understanding of colonization and dissemination, but also to the deeper understanding of polymicrobial sepsis development.

Presentation: Tuesday, 7 March 2017 from 19:30 - 19:45 in room 10-11.

172/EKV
RNAseq analysis reveals cell type-specific reprogramming of host and parasite transcriptomes after infection with *Toxoplasma gondii*
I. J. Swierzy[1], U. Händel[2], A. Kaever[3], M. Scharfe[4], M. Jarek[4], D. Schlüter[2], C. Lüder*[1]
[1]*Georg-August-University, Institute for Medical Microbiology, Göttingen, Germany*
[2]*Otto-von-Guericke University, Institute for Medical Microbiology, Magdeburg, Germany*
[3]*Georg-August-University, Department of Bioinformatics, Göttingen, Germany*
[4]*Helmholtz-Center for Infection Research, Department of Genome Research, Braunschweig, Germany*

The question of how an infected host and its pathogen respond to infection is fundamental for understanding host-pathogen interactions. One of the outstanding features of the apicomplexan parasite *Toxoplasma gondii* is to infect a wide variety of different host cell types of mammals, birds and humans. *T. gondii* is therefore a valuable model to study the impact of an intracellular pathogen on different host cells and vice versa.
High-throughput RNA sequencing revealed more than 16,200 host genes that were differentially regulated in murine skeletal muscle cells (SkMCs), cortical neurons and astrocytes, and fibroblasts before or after infection. However, only between 157 (neurons) to 492 genes (SkMCs) differed at least 2-fold in their expression before and after infection. Intriguingly, largely heterogeneous genes were up- and down-regulated in the different host cells after infection, indicating cell type-specific responses of SkMCs, neurons, astrocytes and fibroblasts after infection. Only a few genes were identified that were commonly regulated in two or three host cell types after infection. For example, transcripts of several cell cycle regulators were commonly up-regulated in SkMCs and neurons after infection. Only a few immunity-related genes including chemokines and CD74 were up-regulated in multiple host cell types. The heterogeneous host cell responses coincided with more than 5,400 *T. gondii* genes that were differentially regulated after infection of the different host cell types. Such regulation largely differed quantitatively rather than qualitatively. Remarkably, BAG1, i.e. a major marker for stage conversion to the latent bradyzoite stage of *T. gondii* was more

strongly expressed in neurons and SkMCs than in astrocytes and fibroblasts. Comparison of the transcriptomes from non-infected host cells uncovered common gene expression signatures in SkMCs and neurons opposed to astrocytes and fibroblasts. These genes encode regulators of the host cell cycle and metabolic processes.
Thus, we have uncovered largely heterogeneous host and parasite responses after infection of different host cell types with *T. gondii* and have identified candidates that might initiate parasite stage differentiation in neurons and SkMCs but not astrocytes and fibroblasts.

Presentation: Tuesday, 7 March 2017 from 19:45 - 20:00 in room 10-11.

WORKSHOP 27
Pathogenicity of Gram-positive cocci (FG MP)
07 March 2017 • 18:00 – 20:00

173/MPV
Lactate Dehydrogenase - A metabolic enzyme involved in virulence of *Streptococcus pyogenes*
S. Oehmcke-Hecht[1], J. B. Wichura[1], L. E. Nass[1], S. Mikkat[2], B. Kreikemeyer[1], T. Fiedler*[1]
[1]*Rostock University Medical Centre, Institute for Mecial Microbiology, Virology and Hygiene, Rostock, Germany*
[2]*Rostock University Medical Centre, Core Facility Proteomics, Rostock, Germany*

Background: *Streptococcus pyogenes* (Group A Streptococcus, GAS) is a major human pathogen frequently reported to cause severe disease. Metabolically, GAS belongs to the lactic acid bacteria (LAB) generating lactic acid as major fermentation product. It has been reported that a lactate dehydrogenase (LDH) negative GAS M49 mutant is not hampered in growth in lab media. Here, we analyzed the impact of the ldh deletion on fitness of GAS in infection relevant conditions.
Methods: Fitness in blood and plasma was analyzed in survival assays. Virulence of the mutants was analyzed in a *Galleria mellonella* infection model. SpeB, streptokinase, and kallikrein activity were determined in colorimetric substrate assays. Kininogen degradation was analyzed in Western Blots. Expression of *speB* was analyzed with qPCR. Amounts of *S. pyogens* proteins and surface bound plasma proteins were determined via nanoACQUITY UPLC / SYNAPT G2-S HDMS.
Results: The *ldh* deletion mutant has a decreased survival in human blood and plasma. The *ldh* deletion leads to a complete loss of secreted cysteine protease SpeB activity that can be restored when *ldh* is complemented. As a consequence of the loss of SpeB activity, the interaction of the mutant strain with the coagulation system is disturbed, which is displayed by an increased plasmin activation and a decreased kallikrein activation and kininogen degradation on the surface of the bacteria after plasma incubation. Furthermore, the *ldh* deletion strain is less virulent in a *Galleria mellonella* infection model.
Conclusions: Our data indicate that LDH is not only involved in fermentative metabolism but also in virulence of GAS, making it another moonlighting protein in GAS.

Presentation: Tuesday, 7 March 2017 from 18:00 - 18:15 in room 5.

174/MPV
***Staphylococcus aureus* 'lipoprotein-like' lipoproteins delay G2/M phase transition in HeLa cells**
M. T. Nguyen*[1], M. Deplanche[2], M. Nega[1], Y. Le Loir[2], L. Peisl[1], F. Götz[1], N. Berkova[2]
[1]*University of Tübingen, Tübingen, Germany*
[2]*Institut National de la Recherche Agronomique, UMR1253 STLO, Rennes, France*

The eukaryotic cell cycle consists of the gap G1 phase characterized by cell growth, the S phase characterized by DNA replication, the second gap G2 phase in which cells are prepared for division, the M phase during which mitosis take place, and the G0 phase when cells can enter a quiescent state. Many bacterial pathogens secrete cyclomodulins that interfere with the host cell cycle. For example, in *Staphylococcus aureus* four cyclomodulins have been described so far that all represent toxins and are secreted into the culture supernatant. In highly pathogenic and epidemic *S. aureus*, a pathogenicity island has been described that contains a cluster of lipoprotein-encoding genes termed *lpl*. The dissemination of Lpl cluster is specific to *S. aureus*, not present in other staphylococcal strains. As the role of *lpl* in virulence and host invasion, a next question is whether Lpl has an effect on delaying the host cell cycle.
Here, we showed an extra function of Lpl on delay host cell cycle. The *lpl* deletion mutant showed a significant lower in G2/M phase transition delay than the wilde type and the complementary strains. We also confirm that *lpl* mutant does not affect the other cell cycle effectors, for example alpha hemolysis or PSM. Moreover, the purified Lpl1, one Lpl representative, both in structure with and without lipid moiety show an effect on G2/M phase delay.

References
[1] Minh Thu Nguyen, et al. *Staphylococcus aureus* 'lipoprotein-like' lipoproteins delay G2/M phase transition in HeLa cells. (Submitted).
[2] Shahmirzadi, Nguyen and Götz. Evaluation of Staphylococcus aureus Lipoproteins: Role in Nutritional Acquisition and Pathogenicity. *Front. Microbiol.* 2016
[3] Minh Thu Nguyen and Friedrich Götz. Lipoproteins of Gram-Positive Bacteria: Key Players in the Immune Response and Virulence. MMBR. 2016
[4] Minh Thu Nguyen, et al. The νSaα Specific Lipoprotein Like Cluster (lpl) of *aureus* USA300 Contributes to Immune Stimulation and Invasion in Human Cells. *PLoS Pathogens*. 2015

Presentation: Tuesday, 7 March 2017 from 18:15 - 18:30 in room 5.

175/MPV
The small histidine-containing phosphocarrier protein Hpr affects glucose metabolism and virulence in *Staphylococcus aureus*.
A. C. Brausch[1], E. Kirch[1], M. Bischoff*[1], R. Gaupp*[1]
[1]*Saarland University, Medical Microbiology and Hygiene, Homburg/Saar, Germany*

Carbon catabolite repression (CCR) is a common mechanism utilized by pathogenic bacteria to link central metabolism with virulence factor synthesis. In *Staphylococcus aureus*, this linkage is mediated via a number of regulatory molecules such as the catabolite control proteins A and E (CcpA and CcpE), CodY, and the RpiR homologs RpiRb and RpiRc. CcpA, which is one of the major players in CCR in Gram-positive bacteria, is thought to respond to the glycolytic intermediates glucose 6-phosphate and fructose 1,6-bisphosphate via the small histidine-containing phosphocarrier protein Hpr, which upon phosphorylation on serine-46 binds to CcpA to stimulate the interaction with its cognate DNA binding sequences called catabolite responsive

elements. The non-essential protein also forms part of the phosphotransferase system that controls sugar uptake and carbon utilization in Gram-positive and Gram-negative bacteria. However, its impact on virulence factor production and infectivity of *S. aureus* has not been addressed yet.
Here we show that inactivation of *ptsH* (encoding Hpr) in *S. aureus* alters the *in vitro* growth kinetics of the mutant by decelerating its doubling time during the exponential growth phase, and strongly interferes with biofilm formation under static and flow conditions, probably due to a decreased sugar uptake and/or metabolism. When utilized in a murine abscess model, the *ptsH* mutant displayed largely reduced bacterial loads in liver and kidney tissue if compared to mice challenged with the wild type strain. Notably, colony forming units determined in liver tissue of mice challenged with the *ptsH* mutant were also clearly lower than in mice infected with an isogenic *ccpA* mutant, suggesting that HPr exerts an effect on infectivity of *S. aureus* beyond activation of CcpA.
Presentation: Tuesday, 7 March 2017 from 18:30 - 18:45 in room 5.

176/MPV
Inactivation of Rsp shows a new side of *Staphylococcus aureus* virulence leading to Trojan horse phenotype.
S. Das*[1], B. Oesterreich[2], K. Förstner[2], K. Paprotka[1], S. Blättner[1], C. Remmele[3], M. Selle[2], M. Dittrich[3], T. Müller[3], J. Vogel[2], K. Ohlsen[2], T. Rudel[1], M. Fraunholz[1]
[1]*University of Wuerzburg, Chair of Microbiology, Wuerzburg, Germany*
[2]*Institute of Molecular Infection Biology, University of Wuerzburg, Wuerzburg, Germany*
[3]*University of Wuerzburg, Chair of Bioinformatics, Wuerzburg, Germany*

Staphylococcus aureus is a successful human pathogen owing to its battery of virulence factors and their intricate regulation. *S. aureus* is an alternative intracellular pathogen which can escape phagolysosomal fusion and subsequently induces host cell death. In a transposon mutant pool screen followed by insertion site deep sequencing we identified factors that contribute to intracellular toxicity of the pathogen. Thereby we identified a major role for the AraC-type transcriptional regulator Rsp as well as the non-coding RNA SSR42 in cell toxicity. Transcriptome analyses identified an Rsp regulon that mainly consisted of immunomodulatory proteins and toxins directed against leukocytes. Rsp mutant bacteria further demonstrated delayed host cell toxicity. In a murine pneumonia model, Rsp mutants were strongly attenuated in their acute virulence, but were able to form deep abscesses just like the wild type. Together our data suggest that mutation within Rsp or its downstream regulon shift pathogenicity from an acute to a delayed virulence and cause a Trojan horse phenotype whereby *S. aureus* dissemination in the host is fostered by prolonged intracellular localization.

References
[1] Sudip Das, Claudia Lindemann et al. Natural mutations in a *Staphylococcus aureus* virulence regulator attenuate cytotoxicity but permit bacteremia and abscess formation. *PNAS 2016 113 (22) E3101-E3110; doi:10.1073/pnas.1520255113.*
[2] Mark S. Smeltzer, *Staphylococcus aureus* Pathogenesis: The Importance of Reduced Cytotoxicity. *Trends in Microbiology*, Volume 24, Issue 9, September 2016, Pages 681-682, ISSN 0966-842X, http://dx.doi.org/10.1016/j.tim.2016.07.003.

Presentation: Tuesday, 7 March 2017 from 18:45 - 19:00 in room 5.

177/MPV
Inhibition of the transcription termination factor Rho by bicyclomycin affects expression of SaeRS-dependent virulence factor genes in *Staphylococcus aureus*
A. Nagel*[1], P. Nicolas[2], M. Debarbouille[3], C. Guerin[2], S. Michalik[1], M. Depke[1], T. Hertlein[4], A. Hiron[3], M. Gesell Salazar[1], J. Pané-Farré[5], M. van der Kooi-Pol[6], E. Reilman[6], P. Bessières[2], K. Ohlsen[4], M. Hecker[5], T. Msadek[3], J. M. van Dijl[6], U. Völker[1], U. Mäder[1]
[1]*Interfaculty Institute for Genetics and Functional Genomics , Department of Functional Genomics , Greifswald, Germany*
[2]*INRA, Mathématiques et Informatique Appliquées du Génome à l'Environnement, Jouy-en-Josas, France*
[3]*Institut Pasteur, Department of Microbiology, Paris, France*
[4]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*
[5]*University of Greifswald, Institute for Microbiology, Greifswald, Germany*
[6]*University Medical Center Groningen, Department of Medical Microbiology, Groningen, Netherlands*

Introduction: In a large-scale transcriptome study using strand-specific tiling arrays we analyzed *S. aureus* HG001, a derivative of the strain NCTC 8325, under multiple experimental conditions. Data of the tiling array transcriptome analysis [1] revealed a relatively low abundance of antisense RNAs in the *S. aureus* wild type, where they overlap only 6% of the coding genes. As known from previous studies, the transcription termination factor Rho plays a major role in suppressing antisense transcription in *E. coli* [2] and *B. subtilis* [3], and indeed there is a remarkable overall increase in antisense transcription in the absence of Rho in *S. aureus* [1]. Proteome analysis of cytoplasmic and secreted fractions comparing *S. aureus* HG001 and its isogenic Δ*rho* mutant showed significant differences in the abundance of several proteins, namely increased amounts of SaeSR-dependent virulence factors like extracellular adherence protein (Eap) and fibronectin-binding proteins (FnbA and FnbB) in the *rho* mutant.
Objectives: Bicyclomycin (BCM) is an antibiotic, which specifically inhibits Rho and can be used for treatment of Gram-negative bacteria, because Rho is essential for their viability [4]. Here we wanted to analyze if treatment of *S. aureus* wild type cells with BCM would result in the same effects on the expression of virulence genes and occurrence of antisense RNAs as observed in the Δ*rho* mutant.
Material & Methods: *S. aureus* strains were grown in RPMI medium and treated with different concentrations of BCM. For the investigation of secreted proteins supernatant was collected and analyzed by mass spectrometry. The cells were harvested and RNA was extracted.
Results: Growth of *S. aureus* was not affected by BCM treatment. By Northern blot analysis higher levels of SaeSR-dependent transcripts were observed in the presence of BCM in the wild type, which increased with increasing BCM concentrations. Similarly, the secretome analysis revealed higher protein amounts of SaeSR-dependent virulence factors. However, Northern blot and secretome analyses showed that expression levels of most SaeSR-dependent genes in wild type cells treated with 80 µg/ml BCM did not reach those observed in the Δ*rho* mutant.
Conclusion: The absence of Rho in *S. aureus* leads to higher amounts of SaeSR-dependent virulence factors and of antisense RNAs. This can also be observed by treating *S. aureus* wild type cells with BCM. In further experiments we found that the Δ*rho* mutant exhibited increased virulence in a murine infection model. Taken together, these findings suggest that BCM potentially has an impact on staphylococcal virulence.

References
[1] U. Mäder *et al.;* PLOS Genet. 2016; 12(4):e1005962.
[2] J. M. Peters *et al.;* Genes Dev. 2012; 26(23):2621-33.
[3] P. Nicolas *et al.;* Science. 2012; 335(6072):1103-6.
[4] A. Das *et al.;* Proc Natl Acad Sci U S A. 1976; 73(6):1959-63.

Presentation: Tuesday, 7 March 2017 from 19:00 - 19:15 in room 5.

178/MPV

A novel role of a major cell wall glycopolymer in *Staphylococcus aureus* virulence

C. Weidenmaier*[1], S. Wanner[1], J. Schade[1], D. Keinhörster[1], S. E. George[1], C. Wolz[1], J. Kolata[2], B. Bröker[3]
[1]*Medizinische Mikrobiologie und Hygiene, Tübingen, Tübingen, Germany*
[2]*Medical Microbiology UMC, Utrecht, Netherlands*
[3]*Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Greifswald, Germany*

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have been under intensive investigation during the last decade due to their fast epidemic spread and their enormous virulence potential which exceeds that of the traditional hospital-acquired strains (HA-MRSA). Skin and soft tissue infections (SSTIs) account for ninety percent of CA-MRSA infections, ranging from mild skin infections to severe abscesses. We report here a novel mechanism that contributes to the elevated virulence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). CA-MRSA are causing a severe pandemic of mainly skin and soft tissue infections and some of the underlying pathomechansisms and virulence factors have been studied in detail. So far, increased expression of core-genome-encoded virulence determinants, such as phenol-soluble modulins, α-toxin and acquisition of phage-encoded genes have been correlated with virulence of CA-MRSA strains. However, the relative role of virulence factors in CA-MRSA virulence is still debated and several lines of evidence hint to the involvement of additional so far unknown factors. When we analyzed cell wall composition of different *S. aureus* strain backgrounds we discovered that especially highly virulent CA-MRSA exhibited severely increased amounts of cell wall attached teichoic acids (WTA). We have shown before that this major glycopolymer of the staphylococcal cell wall can activate CD4-T cells in an MHC II dependent manner and plays an important role in the development of staphylococcal skin abscesses. Since CA-MRSA are the predominant isolates in staphylococcal skin infections, we compared the capacity of cell wall fractions isolated from highly pathogenic CA-MRSA and less virulent strains to induce abscess formation in a subcutaneous mouse model. Abscess induction indeed correlated strongly with the amount of cell wall incorporated WTA. We found that CA-MRSA specifically up-regulated WTA content (WTAhigh) and thus were more active in inducing abscess formation via a T cell dependent mechanism than cell walls from *S. aureus* strains with a WTAlow phenotype. We could demonstrate that the WTAhigh phenotype depends on an increased expression of an integral WTA transporter gene and thus on an increased capacity of WTAhigh strains to transport the nascent WTA chain to the outside of the bacterial cell prior to incorporation into the cell wall. Interestingly, the up-regulation of WTA transporter expression was a function of increased Agr-activity. Agr is a major virulence regulator and high Agr-activity was a hallmark of the WTAhigh strains we analyzed when compared to WTAlow strains. Taken together, we present here a novel concept of staphylococcal virulence that is based on the increased biosynthetic activity of a major staphylococcal cell wall polymer.

Presentation: Tuesday, 7 March 2017 from 19:15 - 19:30 in room 5.

179/MPV

Staphylococcus aureus adaptation: complex adaptation and surviving starvation

C. Liang*[1], A. Christof[1,2], M. Yang[1], A. Giesel[1,3], P. Martin[3], S. Fuchs[3,4], C. Wolz[2], P. Jan[3], T. Dandekar*[1]
[1]*Biozentrum, Universität Würzburg, Bioinformatik, Würzburg, Germany*
[2]*University of Tübingen, Medical Microbiology and Hygiene, Tübingen, Germany*
[3]*Institute of Microbiology, Ernst-Moritz-Arndt University Greifswald, Greifswald, Germany*
[4]*Robert Koch Institut, FG13; Haus D; Raum 02.007, Wernigerode, Germany*

Question: *Staphylococcus aureus* (*S. aureus*) infection is clinical important, its multi-resistance forms are one of the main challenges in hospital treatment of chronic and severe infections. A key question is *S. aureus* ability to survive and how to adapt for different stresses and environments.
Methods: We systematically collected and analyzed a large collection of different *S. aureus* strains with the aim to improve therapeutic strategies and general understanding of its general adaptation and evolutionary potential (Liang et al., 2016). Flux analysis based transcriptome data and proteome data helps to reveal the crucial regulations and adaptations to different environmental stresses. Boolean modeling and semi-quantitative dynamic modeling were applied to study the interactions.
Results and Conclucsion: The pan-complexome of *S. aureus* includes central metabolic complexes under exponential growth which may switch to other complexes (or exchange components) in stationary phase to serve intermediate metabolism (e.g. pyruvate dehydrogenase), amino acid homostasis, as well as dealing with antibiotic or environmental stresses (Cecil et al., 2015). Strain-specific complexes promote strain evolution for instance in wall teichoic acid metabolism as exemplified by strain PS187 (Winstel et al., 2013), and regulation includes a number of rapidly changing interactions such as for quorum sensing and its regulation (Audretsch et al., 2013). We modeled and tested the adaptation of amino acid metabolism by CodY and regarding branched chain amino acid metabolism as this is part of the stringent response which allows for instance survival after phagocytosis (Geiger et al., 2012), we identified new regulatory interactions ,not yet reported in literature. The structure and adaptation information helps to better understand adaptation under such challenging conditions and the complex processes involved. Transcription data are helpful to study gene co-expression to reveal more about protein complexes and regulation events including growth effects.

References

[1] Audretsch C, et al., Mol Biosyst. 2013, 9(11):2665-80.
[2] Cecil A, et al., 2015, 305(1):96-109.
[3] Liang C, et al., 2016, 4, 8.
[4] Winstel V, et al., Nat Commun. 2013;4:2345.
[5] Geiger T, et al., PLoS Pathog. 2012;8(11):e1003016.

Presentation: Tuesday, 7 March 2017 from 19:30 - 19:45 in room 5.

180/MPV

Glycosylated plasmin-sensitive protein Pls in methicillin-resistant Staphylococcus aureus (MRSA) promotes biofilm formation

J. Eikmeier*[1], I. Bleiziffer[1], G. Pohlentz[2], K. McAulay[3], G. Xia[4], M. Hussain[2], A. Peschel[5], S. Foster[3], G. Peters[2], C. Heilmann[1]

Question: Until recently, the inability of bacteria to glycosylate proteins has been considered a dogma. Now it is widely accepted that bacteria can glycosylate proteins and most bacterial glycoproteins identified to date are virulence factors of pathogenic

bacteria, i.e. adhesins and invasins. However, the impact of protein glycosylation on the major human pathogen *Staphylococcus aureus* remains incompletely understood. Therefore, we aimed to identify *S. aureus* surface glycoproteins, analyze the underlying glycosylation machinery and characterize the function of the glycosyl modifications.
Methods: To study protein glycosylation in staphylococci, we analyzed lysostaphin lysates of methicillin-resistant *Staphylococcus aureus* (MRSA) strains by SDS-PAGE, subsequent periodic acid-Schiff"s staining, and mass spectrometry.
Results: We found that the plasmin-sensitive surface protein Pls is a post-translationally modified glycoprotein. *pls* is encoded by the SCC*mec* type I in MRSA and has been demonstrated to be a virulence factor in mouse septic arthritis. In a search for glycosyltransferases, we identified two open reading frames encoded downstream of *pls* on the SCC*mec* element, which we termed *gtfC* and *gtfD*. Expression and deletion analysis revealed that both *gtfC* and *gtfD* mediate glycosylation of Pls. Additionally, the recently reported glycosyltransferases SdgA and SdgB are involved in Pls glycosylation. Glycosylation occurs at serine residues in the Pls SD-repeat region and modifying carbohydrates are *N*-acetylhexosaminyl residues. Functional characterization revealed that Pls can confer increased biofilm formation, which seems to involve two distinct mechanisms. The first mechanism depends on glycosylation of the SD-repeat region by GtfC/GtfD and probably also involves eDNA, while the second seems to be independent of glycosylation as well as eDNA. Other previously known Pls properties are not related to the sugar modifications.
Conclusions: In conclusion, Pls is a glycoprotein and Pls glycosyl residues can stimulate biofilm formation. Thus, sugar modifications may represent promising new targets for novel therapeutic or prophylactic measures against life-threatening *S. aureus* infections.

Presentation: Tuesday, 7 March 2017 from 19:45 - 20:00 in room 5.

WORKSHOP 28
The enteric microbiota and its influence on the host (FG GI / FG PW)
07 March 2017 • 18:00 – 19:30

181/GIV
A low-abundance bile acid acts as a positive regulator of the antimicrobial program of the terminal ileum.
S. Tremblay[1], G. Romain[1], M. Roux[1], X. L. Chen[1], K. Brown[2], D. Gibson[2], S. Ramanathan[1], A. Menendez*[1]
[1]*University of Sherbrooke, Pediatrics, Immunology, Sherbrooke, Canada*
[2]*University of British Columbia-Okanagan, Biology, Kelowna, Canada*

Background and Question: Intestinal epithelial cells play a critical role in gut homeostasis. One of their major mechanisms is the synthesis and secretion of antimicrobial peptides and proteins, which control the number and composition of the intestinal microbiota and protect from infections. Recent data have implicated the nuclear bile acid receptor FXR in the immunity of the small intestine. We hypothesized that bile acids contribute to the immunological homeostasis of the gut through the global regulation of intestinal AMPP expression.
METHODS: *Ex vivo* intestinal explants were treated with several bile acids to evaluate their impact on the synthesis of antimicrobial peptides. C57BL/6 mice fed with a chenodeoxycholic acid (CDCA)-supplemented diet for 16 hours were used to determine the effect of CDCA on antimicrobial peptides synthesis *in vivo*. Alterations in the immune cell populations of the intestinal mucosa were also studied using FACS. Changes in intestinal microbial populations were followed by 16S qPCR. Susceptibility of CDCA-fed animals to enteric infections was tested with the bile-resistant pathogens *C. rodentium* and *S. typhimurium*.
Results: Intestinal explants treated with the low-abundants bile acid CDCA demonstrated a significant and consistent up-regulation of antimicrobial peptides expression. CDCA feeding also increased the base-line synthesis of intestinal antimicrobial peptides *in vivo*; it also had an effect on the mucosal immune cell populations, inducing a decrease in the abundance of macrophages (CD68+) and neutrophils (Ly6G+) and an increase in the percentage of B cells (IgGκ+). Gene expression analyses using DNA microarrays revealed an anti-inflammatory gene expression pattern in the ilea of CDCA-fed mice. Feeding with CDCA promoted a significantly faster clearance of *C. rodentium* and limited the establishment of systemic *S. typhimurium* infection.
Conclusions: Our results demonstrate the existence of a novel mechanism for the control of intestinal antimicrobial peptides expression and reveal a new aspect of the antimicrobial nature of bile acids *in vivo*. The regulation of intestinal antimicrobial peptides synthesis by bile acids is physiologically relevant and seems to facilitate resistance to enteric pathogens, which has the potential to be exploited in therapeutic applications.

Presentation: Tuesday, 7 March 2017 from 18:00 - 18:15 in room 7-8.

182/GIV
Co-infection and pre-infection effects on eukaryotic cell line response to the Cag Type IV secretion system of *Helicobacter pylori*
A. F. Zeitler[1], K. H. Gerrer[1], R. Haas[1], L. F. Jiménez-soto*[1]
[1]*Max von Pettenkofer-Institute, Munich, Germany*

Since its discovery, the bacterium *Helicobacter pylori* has been associated with gastric pathologies. Several of its pathogenicity mechanisms damage eukaryotic cells in *in vitro* conditions and this has been extrapolated to damages to the gastric tissue. The best known cytotoxins from *H. pylori* are the Vacuolating cytotoxin A, VacA, and the product of the cytotoxin associated gene A, CagA. CagA is injected into the cytoplasm of the host cell via the Cag Type IV secretion system (T4SS), which consists of 29 to 32 protein components. A fully functional Cag T4SS is able not only to inject CagA, but it induces the production of inflammatory cytokines thought to be responsible for the recruitment of neutrophils to the gastric mucosa. In nature we can find strains containing the CagA gene, called type I strains, and strains without it (Type II strains). Equally important is the fact that human gastric mucosa can be colonized by several strains. With the aim to investigate the effect of multiple strain infections in *in vitro* assays, we have performed co- and pre-infection experiments using several wild type strains. Our results show that both effects associated with a functional Cag T4SS (CagA translocation and IL-8 production) are reduced when cells have been exposed previously to other *H. pylori* strains[1, 2]. If the *in vitro* data can be extrapolated to the *in vivo* situation, it could mean that infection with multiple strains will influence the toxic effects of the oncogenic toxin CagA and the outcome of gastric diseases associated with severe inflammation caused by *H. pylori*. Recent publications showing a negative association between the eradication (or lack) of *H. pylori* and esophagus cancer or asthma in children are questioning the necessity for a general eradication of *H. pylori* from the human host. We hope that through our studies, a deeper understanding about the probably beneficial influence of multiple strain infections and the virulence of *H. pylori* can be acquired. Such information may help to evaluate the risk for each individual and better predict the kind of medical treatment needed based on the individual´s specific colonization status and disease.

References
[1] Zeitler AF, Gerrer KH, Haas R, Soto LF. Optimized semi-quantitative blot analysis in infection assays using the Stain-Free technology. Journal of microbiological methods 2016.
[2] Jimenez-Soto LF, Clausen S, Sprenger A, Ertl C, Haas R. Dynamics of the Cag-type IV secretion system of Helicobacter pylori as studied by bacterial co-infections. Cell Microbiol 2013.

Presentation: Tuesday, 7 March 2017 from 18:15 - 18:30 in room 7-8.

183/GIV

PrsA2 of *Clostridium difficile* is an active parvulin-type PPIase and influences sporulation and germination

C. Ünal*[1], M. Berges[1,2], C. Priebe[1], C. Schiene-Fischer[3], G. Fischer[3], D. Jahn[1,2], M. Steinert[1,4]
[1]*Technische Universität Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany*
[2]*Technische Universität Braunschweig, BRICS, Braunschweig, Germany*
[3]*Universität Halle-Wittenberg, Institut für Biochemie und Biotechnologie, Halle/Saale, Germany*
[4]*Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany*

Clostridium difficile is the main cause for antibiotic associated diarrhea, and has become a major burden for the health care systems of industrial countries. While two large glucosylating toxins are the main virulence factors, the contribution of others to disease development and progression are only insufficiently studied. Peptidyl-proly-*cis/trans*-isomerases (PPIases) constitute an interesting class of proteins, since they assist protein folding, localization and transport. Moreover, many PPIases have been described in the context of virulence. Accordingly, the putative parvulin-type PPIase CD630_35000 of *C. difficile* with a predicted extracellular location and the highest sequence homology to the virulence associated PrsA2 of *L. monocytogenes* was chosen for detailed characterization.
CD630_35000 (*CdprsA2*) was cloned into the expression vector pSSBM106. Eight highly conserved amino acids in the catalytic cleft of CdPrsA2 were replaced by alanines using site directed mutagenesis. Wild type CdPrsA2 and its mutants were recombinantly produced in *B. megaterium*. Further on, a PrsA2 deficient mutant was generated in the *C. difficile* 630*Δerm* background using the ClosTron Technology, and analyzed with respect to its spore forming capacity, germination and resistance to bile acids.
PrsA2 and its site directed mutants could be produced in good yields and purity. This allowed the identification of catalytically active amino acids as well as the substrate specificity of PrsA2. The PrsA2-defficient mutant showed reduced rates of, sporulation and germination as well as increased bile acid resistance when compared to the wild type.

Presentation: Tuesday, 7 March 2017 from 18:30 - 18:45 in room 7-8.

184/PWV

Human commensals producing a novel antibiotic impair pathogen colonization

A. Zipperer*[1], M. Konnerth[2], C. Laux[1], A. Berscheid[1], D. Janek[1], C. Weidenmaier[1], M. Burian[1], N. Schilling[2], C. Slavetinsky[1], M. Marschal[1], M. Willmann[1], H. Kalbacher[1], B. Schittek[1], H. Brötz-Oesterhelt[1], S. Grond[1], A. Peschel[1], B. Krismer[1]
[1]*Interfakultäres Institut für Mikrobiologie und Infektionsmedizin Tübingen, Tübingen, Germany*
[2]*University of Tübingen, Institute for Organic Chemistry, Tübingen, Germany*

The vast majority of systemic bacterial infections is caused by facultative, often antibiotic-resistant pathogens colonizing human body surfaces. Nasal carriage of *Staphylococcus aureus* predisposes to invasive infection, but the mechanisms permitting or interfering with pathogen colonization have remained largely unknown. Whereas soil microbes are known to compete by production of antibiotics, such processes have rarely been reported for the human microbiota. We show that nasal *Staphylococcus lugdunensis* strains produce lugdunin, a novel thiazolidine-containing cyclic peptide antibiotic prohibiting colonization by *S. aureus,* and a rare example of a non-ribosomally synthesized bioactive compound from human-associated bacteria. Lugdunin is bactericidal against major pathogens, effective in animal models, and not prone to resistance development. Importantly, human nasal colonization by *S. lugdunensis* was associated with a significantly reduced *S. aureus* carriage rate suggesting that lugdunin or lugdunin-producing commensals could be valuable for preventing staphylococcal infections. Moreover, human microbiota should be considered as a source for new antibiotics.

Presentation: Tuesday, 7 March 2017 from 18:45 - 19:00 in room 7-8.

185/PWV

Pathogenicity Mechanisms of *Enterococcus faecalis* in Chronic Intestinal Inflammation

I. Lengfelder*[1], I. Sava[1], S. Ocvirk[1], J. Hansen[2], B. Sartor[2], D. Haller[1,3]
[1]*Technische Universität München, Nutrition and Immunology, Freising, Germany*
[2]*Universtity of North Carolina, Division of Gastroenterology and Hepatology, Chapel Hill, United States*
[3]*Technische Universität München, ZIEL Institute for Food and Health, Freising, Germany*

Introduction: *Enterococcus faecalis* (*E.f*) is a commensal of the intestinal core microbiota harboring several virulence factors, which highlight its role as opportunistic pathogen. This dualistic character is supported by evidence linking *Enterococcus* spp. to the pathogenesis of inflammatory bowel diseases.
Objective: Our aim is to dissect disease-relevant microbe-host interactions particularly related to adaptive mechanisms of *E.f* in response to the host-derived inflammatory milieu.
Materials & methods: We are using the IL10-/- mouse, a chronic colitis model, monoassociated with wild-type (Wt) *E.f* and deletion mutants to dissect disease relevant microbe-host interactions. Bacterial transcriptome in response to inflammation was assessed via RNA sequencing. *E.f* in a complex environment was studied in gnotobiotic mice colonized with a colitogenic simplified human microbial consortium (SIHUMI).
Results: RNA sequencing of *E.f* identified 98 up- and 142 down-regulated genes under inflammation. Surprisingly, expression levels of most *E.f* virulence genes did not undergo substantial alterations (Ocvirk et al., 2015), whereas several genes relevant for bacterial fitness were differentially expressed. Among the highest upregulated genes, the major facilitator superfamily transporter (Mfs) and several genes controlling ethanolamine utilization were identified. Adequate bacterial deletion mutants were generated and analyzed in monoassociated IL10-/- mice. Absence of ethanolamine utilization resulted in a minor reduction of bacterial colitogenic activity, pro-inflammatory cytokine secretion of reactivated MLN cells and IFNγ mRNA expression in colonic tissue. Bacterial colonization density was not influenced. IL10-/- mice colonized with Mfs mutant showed similar levels of intestinal pathology and bacterial colonization compared to Wt *E.f* colonized mice. However, IFNγ and TNF expression and secretion was induced in colonic tissue as well as in reactivated MLN cells, respectively, suggesting a more aggressive inflammatory phenotype.
Abundance levels of *E.f* in a complex colitogenic bacterial environment (SIHUMI) ranged between 2% to 4%. Colonization of IL-10-/- mice with SIHUMI in the absence of *E.f* still induced inflammation, suggesting that the colitogenic activity of low abundant bacteria is compensated by *Ruminococcus gnavus*, *Bacteroides vulgatus* or *Escherichia coli*.

Conclusion: Transcriptional profiling of colitogenic bacteria revealed an adaptive response towards intestinal inflammation. Despite their considerable upregulation in inflamed hosts, deletion of Mfs and ethanolamine utilization genes had no major influence on bacterial colonization and colitogenic activity. Colonization of IL10-/- mice with a minimal bacterial consortium demonstrated that the colitogenic activity of *E.f* is compensated by other members of the SIHUMI consortium.

Presentation: Tuesday, 7 March 2017 from 19:00 - 19:15 in room 7-8.

186/PWV

Identification of disease-relevant bacterial signatures in gnotobiotic IL-10 deficient mice using fecal samples from IBD patients undergoing hematopoietic stem cell transplantation

A. Metwaly*[1], L. F. Buttó[1], N. Waldschmitt[1], I. Lagkouvardos[2], A. M. Corraliza[3], A. Mayorgas[3], M. Martinez-Medina[4], M. Allez[5], J. Panes[3], A. Salas[3], D. Haller[1]
[1]*Technical University Munich (TUM), Chair of nutrition and immunology, Freising, Germany*
[2]*ZIEL-Institute for Food and Health, Freising, Germany*
[3] *Instituto de Investigaciones Biomédicas de Barcelona CSIC, IDIBAPS, CIBERehd Spain, Department of Experimental Pathology, Barcelona, Spain*
[4]*Laboratory of Molecular Microbiology, Universitat de Girona, Girona, Spain*
[5]*APHP, Hôpital Saint Louis,INSERM UMRS 1160, Paris Diderot, Sorbonne Paris-Cité University, Department of Gastroenterology, Paris, France*

Introduction: Imbalanced microbial composition has been linked to the pathogenesis of inflammatory bowel disease (IBD). Hematopoietic stem cell transplantation (HSCT) proved to be successful in inducing remission in a subset of severe, highly refractory Crohn's disease (CD) patients, possibly by erasing immune responses against the gut microbial ecosystem. Gnotobiotic mouse models colonized with human microbiota have brought insights into the functional and mechanistic aspects of host-microbe interactions.
Objectives: The aim of this study was to assess the functional role of microbiota signatures associated with different disease-states. We used the fecal microbiota from CD patients treated with HSCT to colonize an IBD-relevant mouse model.
Patients & Methods: High-throughput 16S rRNA gene amplicon sequencing was performed on (n=147) fecal samples collected from (n=13) healthy donors and (n=31) HSCT-treated CD patients. Germ-free (GF) wild-type (WT) and IL10-/- mice (129 Sv/Ev; n=12 mice/group) were colonized with fecal microbiota from CD patients before and after HSCT at different disease states. Selection of CD patients for transplantation into GF mice was based on clinical and endoscopic disease activity; including paired patient samples collected under remission or relapse.
Results: Microbiota profiling showed a significantly reduced microbial diversity in patients compared with healthy controls. Patients in remission showed higher microbial diversity compared to patients in relapse . Patients with fistulating or ileal phenotype had the least diverse ecosystems. High level of inter-individual variation in the intestinal microbiota of healthy and diseased donor samples was observed. Despite an incomplete transfer of donor microbiota with a 20-40% loss of species-level taxa after transplantation, humanized mice reflected the dysbiotic features of their respective human donors, indicated by richness and diversity measures. Histopathological evaluation showed moderate to severe inflammation in colon and cecum of the IL10-/- mice associated with microbiota obtained from patients in relapse. In contrast, IL10-/- mice associated with microbiota from patients in remission remained disease-free. To validate the phenotype transfer even in the presence of multiple inoculations, we gavaged the mice three times with donor microbiota during the first week of colonization. Remission-associated mice showed higher species richness but still remained disease free, while relapse-associated mice developed enhanced inflammation measured at the level of fecal complement C3 concentrations. Endpoint microbial composition remained similar, regardless of the number of inoculations and F1 generations of mice displayed a stable engraftment of human microbiota.
Conclusion: Transfer of patient-derived fecal microbiota can mimic the disease phenotype (remission vs. relapse after HSCT) in gnotobiotic IL10-/- mice. Bacterial composition, not the number of species is responsible for disease initiation. We used humanized mice as a tool to identify bacterial signatures associated with disease status in IBD patients treated with HSCT.

Presentation: Tuesday, 7 March 2017 from 19:15 - 19:30 in room 7-8.

WORKSHOP 29
Epidemiology, diagnostic, surveillance and prevention of multi drug resistant organisms (FG PR / StAG HY)
07 March 2017 • 18:00 – 19:30

187/HYV

Improved infection control strategies and quality of care for patients affected by multi-drug resistant bacteria using patients` experiences

A. Kossow*[1,2], F. Kipp[3], A. Mellmann[2], C. Hornberg[1]
[1]*University of Bielefeld, School of Public Health, Bielefeld, Germany*
[2]*University of Münster, Institute of Hygiene, Münster, Germany*
[3]*DRK Kliniken Berlin, Institute of Hygiene, Berlin, Germany*

Question: Multi-drug resistant organisms (MDRO) are a growing public health challenge, burdening health care systems with increased costs. Whereas direct and indirect costs can be objectively measured, intangible costs, i.e. fear, stress and reduced quality of life, are borne by the patients. Patients colonized or infected by MDRO are subjected to additional infection measures like contact isolation and the use of gowns, gloves and face masks.1 A widely recognized decreased quality of care may lead to insecurities among the patients and a need of information. The patients" point of view can reveal otherwise undetected factors that hinder high quality of care and adequate information about MDRO. As patients participate in all medical sectors interfaces, their experiences can help to improve infection control strategies to prevent MDRO across different sectors.
Methods: We conducted 14 semi-structured interviews with patients infected or colonized by Methicillin resistant *Staphylococcus aureus*, Vancomycin-resistant Enterococci, and multi-drug resistant Gram-negative bacteria. The study was set within the context of health service research, focusing on patient orientation. The interview guide was based on Picker"s principle of patient-centered care2 with emphasis on quality of care and patient information. Interviews were analyzed using qualitative content analysis.
Results: The interviews revealed that patients" experiences varied with social background and the presence of heterogeneous underlying diseases playing a role. Information can be improved, as there are several uncertainties, i.e. considering the contact to relatives. Protective clothing and inadequate implementation of hygiene measures have a strong effect on the experience of the quality of care. Patients see understaffing as the reason for constraints. The general practitioner (GP) is often seen as the physician of trust.
Conclusions: Adequate information and adequate implementation of hygiene measures can lead to a better quality of care and greater patient satisfaction. Since the GP is seen as a person of

trust, his role in coordinating patient care should not be underestimated.

References

[1] Hubner NO, Hubner C, Kramer A. [Economic aspects of the management and control of MRSA]. Gesundheitswesen 2009; 71(11): 771-6.
[2] Gerteis M. Through the patient's eyes: understanding and promoting patient-centered care. 1. ed. San Francisco: Jossey-Bass; 1993.

Presentation: Tuesday, 7 March 2017 from 18:00 - 18:15 in room 6.

188/PRV

Monitoring the microevolution of OXA-48-producing *Klebsiella pneumoniae* ST147 in a hospital setting by SMRT sequencing

A. E. Zautner*[1], B. Bunk[2], Y. Pfeifer[3], C. Spröer[2], U. Reichard[4], H. Eiffert[1], S. Scheithauer[4], U. Groß[1], J. Overmann[2], B. Wolfgang[1]
[1]*University Medical Center Göttingen, Medical Microbiology, Göttingen, Germany*
[2]*DSMZ, Braunschweig, Germany*
[3]*Robert Koch-Institut, Wernigerode, Germany*
[4]*University Medical Center Göttingen, Infection Control and Infectious Diseases, Göttingen, Germany*

Carbapenemase-producing *Enterobacteriaceae* have emerged as a major global health threat. Spread of carbapenemases occurs either by transmission of successful clonal lineages, or by horizontal gene transfer of carbapenemase gene carrying plasmids. Currently, OXA-48 is the most frequent carbapenemase in Germany.
We characterized 16 carbapenemase-positive *Klebsiella pneumoniae* isolates that were identified from March 2013 to June 2015 at the Institute for Medical Microbiology, University Medical Center, Göttingen. Performing qPCR analysis, fifteen isolates were *bla*OXA48-positive and a single isolate was *bla*NDM-1-positive. Whole genome sequencing was applied on all isolates using the PacBio *RSII* platform. MLST analysis identified eight isolates of sequence type ST147; other sequence types were ST11, ST101, ST23, ST15 and ST395. Core genome MLST revealed that the eight ST147 isolates are the same cgMLST sequence type and thus of clonal origin. These data are in agreement with results from PFGE analysis that show identical patterns for these ST147 isolates. Pairwise MAUVE alignment demonstrated only 1-25 SNPs in the eight S147 isolates and CSIPhylogeny was used to visualize microevolution of the isolates. Most of the eight ST147 isolates carried four plasmids with sizes of 247 kb, 96.1 kb, 63.6 kb and 61 kb and an extracellular element with an incomplete prophage. The *bla*OXA-48 gene was localized on a 63.6 kb IncL/M-like plasmid and is part of a composite transposon Tn1999 variant. The applied whole genome analysis allowed a monitoring of *K. pneumoniae* ST147 microevolution during clonal transmission. Further it revealed in addition to the identification of SNPs, several rearrangements of mobile genetic elements and losses of chromosomal and plasmidic regions.

Presentation: Tuesday, 7 March 2017 from 18:15 - 18:30 in room 6.

189/PRV

Molecular tracing of ESBL-/AmpC-producing enterobacteria along the broiler production chain

M. Projahn*[1], K. Daehre[1], A. Friese[1], U. Roesler[1]
[1]*Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany*

Introduction: Enterobacteria, which produce extended-spectrum beta-lactamases (ESBLs) or AmpC beta-lactamases, are an increasing problem in human and veterinary medicine. Previous studies showed that commensal resistant enterobacteria occur on broiler fattening farms and even one-day-old chicks can be affected.
Objectives: The transmission routes of ESBL-/AmpC-producing enterobacteria along the broiler production chain, vertical and/or horizontal, are not jet clarified. Therefore, we investigated seven ESBL-/AmpC-positive broiler parent flocks (A-G), their corresponding eggs and chicks at the hatchery as well as the following fattening flocks.
Materials and Methods: Various specimen from the animals and the environment of the respective production steps were taken and analyzed concerning ESBL-/AmpC-producing enterobacteria using MALDI-TOF, Disk Diffusions Tests, (real-time) multiplex PCR and sequencing approaches. To identify possible transmission routes and epidemiological relationships, further investigations like pulsed-field gel-electrophoresis (PFGE) and whole genome analyses (WGA) will be done.
Results: In total, we analyzed 36 samples from the parent flocks of which 24 were positive for ESBL-/AmpC-producers of different species (*E. coli, E. fergusonii*) and *bla* resistance genes (*bla*TEM, *bla*CMY, *bla*CTX-M). At the hatchery only 0.6% of the investigated samples (n=1,571) from the eggs and the environment were tested positive whereas at the fattening farm environmental samples from all seven flocks and animal samples from six out of seven flocks were confirmed to be ESBL-/AmpC-positive. Additionally, we determined isolates with comparable genotypes (species, phylogroup, resistance gene) at different production steps for some broiler flocks. *E. coli* isolates showing identical PFGE-pattern and harboring a CTX-M-1 gene were found in the parent flock B and on the outer eggshell surfaces in the hatchery. In flock C we detected comparable isolates (phylogroup F, CMY-2) in both, the parent flock and the fattening flock, even before the chicks arrived and during the whole fattening period. Flock E and F, which were fattened consecutively in the same house, were positive for *E. coli* of phylogroup B1 harboring an SHV-12 gene, respectively.
Discussion: We could verify a pseudo-vertical transfer of ESBL-/AmpC-producing enterobacteria from the parent flock to the hatchery via contaminated outer egg surfaces using PFGE analyses. On the other hand we could also determine indications for either an early contamination of the recently hatched chicks in the hatchery or an entry of the resistant bacteria due to contaminated farm environment. Taken together, a combination of different transmission events along the broiler production chain is very likely and should be considered for intervention strategies.

Presentation: Tuesday, 7 March 2017 from 18:30 - 18:45 in room 6.

190/PRV

Confrontation of nosocomial pathogens with their natural enemy – biofilm reduction and synergistic effects with antibiotics using bacteriophages

S. Latz[1], M. Jansen[1], A. E. Maennling[1], D. Ropohl[1], K. Ritter[1], H. P. Horz*[1]
[1]*RWTH Aachen University Hospital, Institute of Medical Microbiology, Aachen, Germany*

Introduction: Bacteriophages (phages) represent a potential alternative for combating multi-drug resistant bacteria. Because of their narrow host range and the ever emergence of novel pathogen variants the continued search for phages is a prerequisite for optimal treatment of bacterial infections. Previously we isolated novel phages with lytic activity against clinical isolates of the ESKAPE group (i.e. Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter cloacae).
Objectives: In this study we performed an in depth characterization of novel phages regarding genomic content, growth/kill dynamics, biofilm reduction as well as synergistic effects when applied together with antibiotics.
Materials & methods: Novel phages were de-novo sequenced on a MiSeq, followed by genomic analysis using a variety of bioinformatics tools. The host spectrum was determined based on distinct clinical bacterial isolates differentiated via repetitive intergenic consensus sequences. Phage morphology was determined via electron microscopy using two different staining methods. Growth/kill curves with and without antibiotics, (i.e. Meropenem, Ciprofloxacin and Colistin) and with varying multiplicities of infections (MOI), were measured for a period of 16 hours. The effect of phages on newly established biofilms was investigated both at 37°C and at room temperature (RT) based on measuring optical density and ATP levels.
Results: In total 23 different phages isolated against gram-negative bacteria belonging to the ESKAPE group were analyzed. Genomic analysis allowed the identification of genes required for integration into the host genome which enabled the exclusion of undesired lysogenic phages. The genome similarity of remaining (14 lytic) phages to those already deposited in public databases ranged from only 50% for A. baumannii phages up to 98% for P. aeruginosa phages. Based on optical density measurement a biofilm reduction up to 95% could be determined for one of four lytic K. pneumoniae phages. In addition, the activity of P. aeruginosa in biofilms measured by ATP levels could be reduced by 80% with one of three tested phages. Biofilm reduction was always higher at 37°C compared to RT. A synergistic effect was observed with two Acinetobacter phages and Meropenem, leading to a complete elimination of A. baumannii isolates, which were otherwise resistant to Meropenem alone. Remarkably, this synergy was observed even with a MOI as low as 0.01. No such effect was observed with Ciprofloxacin or Colistin.
Conclusion: We have now a number of well-characterized natural phages available as promising alternatives or complementation of antibiotics. Phages are currently being tested in vivo using Galleria mellonella as an infection model, the data of which also will be presented.

Presentation: Tuesday, 7 March 2017 from 18:45 - 19:00 in room 6.

191/PRV

Genomic epidemiology of ESBL-producing *E. coli* from wild migratory birds in Pakistan

M. Mohsin*[1], S. Raza[1], S. Guenther [1]
[1]*Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan*

Question: The increased presence of ESBL producing Enterobacteriacae in humans, animals, and their surrounding environments is of global concern. In this study, we report on the molecular characterization through whole genome sequencing (WGS) of ESBL-producing *E. coli* from wild migratory birds in Pakistan.
Methods: ESBL producing *E. coli* from fecal samples of wild migratory birds were identified using cultivation on CHROMagar ESBL plates followed by confirmation using VITEK-2 AST system. For genetic characterization, WGS was carried out on Illumina MiSeq instrument (Illumina, USA). *In-silico* analysis was performed on web service of the Center for Genomic Epidemiology. Single nucleotide polymorphism- based phylogeny was determined by the software package Harvest Suite and phylogenetic tree was prepared using iTol. Differences in number of SNPs were calculated using MEGA 7 pairwise analysis of core genomes.
Results: A total of 27 (27%) ESBL-producing *E. coli* strains were recovered from fecal swabs of 100 wild migratory avian species distributed across 04 wetland habitats in Pakistan. Ten different sequence types were detected of which ST10 (4/27), ST4720 (3/25) and ST1421 (3/25) were present. All isolates carried the beta-lactam gene *bla*CTX-M-15, 22/30 carried aminoglycosides resistance genes *strA* and *strB*, 14/30 *aac(6')-lb-cr*, tetracycline (*tetB*) (22/30) and 11/30 *qnrS1*. Antibiotic resistance was associated with IncFIA, IncFIB, IncI2 and IncY replicon plasmids. Phylogenetic analysis showed a very high genetic similarity among 06 ESBL-*E. coli* isolates with low numbers of SNPs.
Conclusion: Our findings indicate a great genetic distribution of ESBL- *E. coli* in these transboundary migratory birds. Clonal dissemination of certain clinically relevant phylogenetic lineages underline the zoonotic potential of clinically relevant multiresistant bacteria.

Presentation: Tuesday, 7 March 2017 from 19:00 - 19:15 in room 6.

192/PRV

Faecal carriage of CTX-M type extended spectrum beta lactamase-producing Enterobacteriaceae among street children in Mwanza city, Tanzania

N. Moremi*[1,2], H. Claus[1], U. Vogel[1], S. E. Mshana[2]
[1]*Institut für Hygiene und Mikrobiologie, Wuerzburg, Germany*
[2]*Catholic University of Health and Allied Sciences, Mwanza, Tanzania*

Introduction: The rate of healthcare-associated infections caused by extended spectrum beta lactamase (ESBL)-producing Enterobacteriaceae (EPE) has been increasing not only in local settings but also globally. Information regarding the spread of EPE in community settings in Tanzania is limited. This study analyzed the prevalence of EPE in Tanzanian street children with rare contact to healthcare facilities.
Methods and Materials: Between April and July 2015, 107 street children, who live in urban Mwanza were enrolled in a study. Demographic and other relevant data were collected. Single fresh stool samples were collected from each child and analyzed for EPE. Beta lactamase genes and the multilocus sequence type were characterized. Data were analyzed using STATA-13 software.
Results: The mean age of the enrolled children was 14.2 ± 3.6 years. Among 107 children analyzed, intestinal carriage of EPE was found in 34 (31.8%, 95% CI; 22.7-40.3). The carriage of ESBL-producing-*Escherichia coli* (*E. coli*) was significantly higher than ESBL-producing-*Klebsiella pneumoniae* (*K. pneumoniae*) (28% vs. 5.6%, $p<0.001$). Out of 36 isolates, 36 (100%), 35 (97%), 25 (69%) and 16 (44%) were found to be resistant to tetracycline, trimethoprim-sulfamethoxazole, ciprofloxacin and gentamicin respectively. *Bla*CTX-M-15 was detected in 75% (27/36) of ESBL isolates. Eighteen different *E. coli* multilocus sequence types (ST) were observed of which ST131 (5/30), ST10 (3/30), ST448 (3/30) and ST617 (3/30) were the most prevalent. Use of local herbs for paramedical purposes

(OR: 3.5, 95% CI: 1.51-8.08, p=0.003) and sleeping on the streets at night (OR: 3.6, 95% CI: 1.44-8.97, p=0.005) were found to be independent predictors of ESBL carriage, whereas there was no association to self-reported use of antibiotics.
Conclusion: We observed a high prevalence of EPE in children with limited access to medical care and *bla*CTX-M-15 was shown to be widely spread. Detection of *E. coli* STs 131, 10, 38 and 648 which are observed worldwide in animals and humans calls for the One Health approach to combat further spread of EPE in the environment. Furthermore, the high rate of self-reported antibiotic use is worrying and demands further studies in the setting of street children.

Presentation: Tuesday, 7 March 2017 from 19:15 - 19:30 in room 6.

SHORT LECTURE 30
Microbial community dynamics in the environment (SL EE)
08 March 2017 • 08:30 – 10:00

193/EEV
Collapse of Soil Microbial Biodiversity after Rapeseed-Glucosinolate Exposure
F. Kovacic*[1], M. Siebers[2], T. Rohr[2], M. Ventura[2], S. Thies[1], M. Ferrer[3], K. E. Jaeger[1,4], M. Berg[5], P. Dörmann[2], M. Schulz[2]
[1]*Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Jülich, Germany*
[2]*Institute of Molecular Physiology and Biotechnology of Plants, University of Bonn, Bonn, Germany*
[3]*Consejo Superior de Investigaciones Científicas, Institute of Catalysis, Madrid, Spain*
[4]*Institute of Bio- and Geosciences IBG-1: Biotechnology, Forschungszentrum Jülich, Jülich, Germany*
[5]*Institute for Organic Agriculture, University of Bonn, Bonn, Germany*

Land plants live with soil bacteria and fungi in intricate communities which are indispensable for survival and growth. The complex interactions between the plant and its microbiome are largely governed by different metabolites. Here, we employed a combination of lipid-fingerprinting, enzyme activity assays, high-throughput DNA sequencing and quantitative proteomics analysis to uncover the dynamics of the bacterial and fungal community structures of the soil after exposure to isothiocyanates, toxic catabolites derived from glucosinolates of rape and other Brassicaceae. We could show a strong impact of isothiocyanates on soil bacteria as RNA sequencing results revealed massive propagation of only a few bacterial taxonomic groups and rapid disappearance of many others, mostly without recovery. Fungi of the Ascomycota and Zygomycota were severely affected or extinguished, demonstrating their sensitivity to rapeseed-derived isothiocyanates. *Trichosporon* species withstood isothiocyanate treatment and finally became dominant. Lipid fingerprinting analysis revealed strong increases in phosphatidic acid contents in soil treated with isothiocyanate indicating the dying biomass. The presence of isothiocyanates in the soil led to enhanced phospholipase and protease activities as confirmed by proteomics studies. These results provide the basis for enhanced soil respiration. Conclusively, plants of the Brassicaceae strongly modify the soil microbial diversity by release of isothiocyanates, leading to extreme losses within the microbial community structure and presumably resulting in severe disturbance of the soil carbon cycle.

Presentation: Wednesday, 8 March 2017 from 8:30 - 8:45 in the Franconia Hall.

194/EEV
Earthworm feeding guild affects the selective activation of fermenters in the earthworm alimentary canal
T. Oppermann*[1], P. S. Depkat-Jakob[1], H. L. Drake[1]
[1]*University Bayreuth, Ecological Microbiology, Bayreuth, Germany*

The earthworm alimentary canal is an anoxic microzone in aerated soils, constituting an oasis for microorganisms capable of anaerobiosis. The feeding habits of the anecic *Lumbricus terrestris* and the endogeic *Aporrectodea caliginosa* are distinct, as the former mostly ingests large amounts of plant litter, whereas the latter mainly ingests mineral soil. Due to these contrasting ecotypes, we hypothesised that fermenters along the two alimentary canals are dissimilar and would yield contrasting processes.
Fermentation products of four sections (crop/gizzard, fore-, mid-, and hindgut) of the alimentary canals of *L. terrestris* and *A. caliginosa* were analyzed. Bacterial 16S rRNA and 16S rRNA gene sequences were obtained by Illumina sequencing.
L. terrestris and *A. caliginosa* specimens emitted H_2 *in vivo*. Profiles of glucose and organic acids along the alimentary canal of both earthworm species were similar in quality, but dissimilar in quantity. Succinate and acetate were the predominant fermentation products, indicative of mixed acid fermentations. The alimentary canal of *A. caliginosa* was characterized by (1) a similar relative abundance of 16S rRNA based phylotypes across all sections, at both gene and transcript levels, (2) *Bacillaceae* and *Bradyrhizobiaceae* being the most abundant taxa in all sections, and (3) a higher relative abundance of *Acidobacteria* in mid- and hindgut compared to crop/gizzard and foregut. In marked contrast, the alimentary canal of *L. terrestris* was characterized by (1) dynamic changes in relative abundance of phylotypes on both gene and transcript level, (2) these dynamics being particularly pronounced at the transcript levels, (3) *Enterobacteriaceae* and *Pseudomonadaceae* dominating the crop/gizzard and foregut, and (4) *Aeromonadaceae* and *Flavobacteriaceae* dominating the mid- and hindgut.
The majority of the detected taxa are capable of facilitating mixed acid fermentations, while some are capable of facilitating denitrification and iron reduction, both processes previously detected in the gut of earthworms. The phylogenetic dissimilarity of the taxa in *L. terrestris* and *A. caliginosa* likely reflects the dissimilar microbiomes of the contrasting ingested material of these earthworms (i.e. mainly litter and mineral soil, respectively), while the detected fermentation products reflect a functional redundancy of the gut microbiota of both earthworm species. H_2, a product of mixed acid fermentation, is emitted *in vivo* by the earthworm and could drive secondary redox processes in aerated soils.

Presentation: Wednesday, 8 March 2017 from 8:45 - 9:00 in the Franconia Hall.

195/EEV
Pronounced bacterial population dynamics in a Northern German gas reservoir connected to production-related technical measures
J. Sameith *[1], C. Gniese[2], T. Lueders[3], M. Schlömann[4], M. Krüger[5]
[1]*Bundesanstalt für Materialforschung und -Prüfung, Biodeterioration and Reference Organisms, Berlin, Germany*
[2]*tba, Berlin, Germany*
[3]*Helmholtz Zentrum München, Institute of Groundwater Ecology, Neuherberg, Germany*
[4]*TU-Berakademie Freiberg, Institute of Biosciences, Freiberg, Germany*
[5]*Bundesanstalt für Geowissenschaften und Rohstoffe, Geomicrobiology, Hannover, Germany*

The microbial population of gas and oil reservoirs is of special interest for industrial applications. The population, however, is confronted with a rather severe change of environmental

conditions during the production process, as pressure and temperature gradually decrease. The application of technical measures to improve or stabilize the hydrocarbon recovery from such biospheres is another factor influencing the microbial population. Therefore, the investigation of the microbial community in such reservoirs and its activity in relation to the production processes is difficult to assess. In this study the natural gas reservoir Schneeren-Husum (Lower Saxony, Germany) was monitored for specific microbial parameters for more than three years in a row. Interestingly, saline formation fluids from two neighboring wells (denoted by Z2 and Z3) differed in various geochemical parameters revealing spatially separated compartments already within one reservoir block. The long-term monitoring showed a high variability of the microbial community, i.e. its size and the distribution of microbial metabolic activities. Our microbiological survey included a close examination of the bacterial community in produced and deep-reservoir (unproduced) formation fluids. A high abundance of sulfate reducers (*dsrA* gene-copy numbers) and sulfate-reduction activity, using various substrates like LMWOA, saccharides, and alcohols, were detected likewise in produced and deep-reservoir fluids. Further, using a broad set of physiological and molecular-biological analysis, this study revealed a pronounced community shift from mainly *Proteobacteria* towards thermophilic spore-forming *Clostridiales* between 2008 and 2011. In summary, our results indicated a predominance of sulfate-reducing prokaryotes under *in situ* reservoir conditions.

Presentation: Wednesday, 8 March 2017 from 9:00 - 9:15 in the Franconia Hall.

196/EEV

Deciphering the bacterial response in the rhizosphere towards different management regimes and short-term herbivory in permanent German grassland

B. Wemheuer[1], C. Scherber[2], R. Daniel[1], S. Vidal[3], F. Wemheuer*[3]
[1]*Institute of Microbiology and Genetics, Department of Genomic and Applied Microbiology, Göttingen, Germany*
[2]*Institute of Landscape Ecology, Animal Ecology and multitrophic interactions, Münster, Germany*
[3]*Department of Crop Sciences/ Georg-August-University Göttingen, Agricultural Entomology, Göttingen, Germany*

Many rhizosphere bacteria are important for plant growth and health. However, the response of these microbes to different grassland management regimes and to above-ground herbivory is still poorly understood. Hence, we investigated the combined impact of herbicide application (against monocots and dicots, respectively), two mowing frequencies (once vs. three times a year), two fertilization treatments (no vs. fertilization) and above-ground herbivory on rhizosphere bacterial communities. For this purpose, a lysimeter experiment was conducted on the GrassMan experimental field, a semi-natural, moderately species-rich grassland site. Following a two-week exposure to herbivory, soil samples were taken from the rhizosphere. Community composition was studied by DGGE as well as large-scale pyrosequencing-based analysis of 16S rRNA gene sequences amplified from extracted environmental DNA. In addition, artificial metagenomes were predicted from obtained 16S rRNA gene data to study bacterial functioning. We recorded significant differences in bacterial community composition with respect to the applied management regimes. Herbicide application resulted in a strong significant response of the bacterial community. Mowing frequencies affected bacterial communities in dicot-reduced plots while fertilizer application impacted bacterial communities in all plots. Although no effect of herbivory was observed, several bacterial genera, such as the diazotrophic genus *Ideonella*, were highly associated with herbivory. Several genes including two genes involved in plant polymer degradation were more abundant in herbivory plots compared to control plots. The unique combination of parameters studied provides exceptional insights into the diversity and ecology of bacterial communities in the plant rhizosphere.

Presentation: Wednesday, 8 March 2017 from 9:15 - 9:30 in the Franconia Hall.

197/EEV

Assess the role of viruses in contaminant biodegradation through metagenomics

L. Deng*[1], J. Feichtmayer[2], C. Griebler[2]
[1]*Helmholtz Zentrum Muenchen, Institute of Virology, Munich, Germany*
[2]*Helmholtz Zentrum Muenchen, Institute of Groundwater Ecology, Munich, Germany*

Viral communities are emerging as fundamental drivers of ecosystems by profoundly shaping microbial populations and processes that go beyond mortality and gene transfer to also include direct manipulation of metabolic pathways integral to host-cell function. Consequently, biogeochemical processes in microbial ecosystems and their potential for novel niche adaptation in response to changing environmental conditions can be understood only when this large dynamic gene pool carried by lytic and temporary viruses is recognized. However, little is known about this gene pool in groundwater ecosystem, especially in the contaminated groundwater.
The newly founded Emmy Noether project aims to elaborate a new perspective, the viral-driven degradation. We present here a powerful toolkit – from the concentration [1] and purification [2] of viral particles to the amplification of the resulting DNA for sequencing preparation [3] – for studying environmental virus communities in the omics era. In addition, Viral-Tagging is a high-throughput method to link wild viruses to specific host cells for screening and sequencing. [4, 5].
These improved tools allow for much greater access to the tagged viral community to study virus-host interactions in complex communities. In addition, they equip us to study viral ecology by quantitatively linking objectively defined environmental viral populations, and their genomes, to their hosts, thus can better elucidate the processes that drive the population structure of virus and their host in nature. We use these new tools studying the viral community in the contaminated groundwater and underline the mechanism of how viruses impacting contaminants degradation through (i) horizontally transfer host metabolic genes related to contaminant degradation, and (ii) specifically lysing key bacterial degraders.

References

[1] John et al (2011) A simple and efficient method for concentration of ocean viruses by chemical flocculation. *Environmental Microbiology Reports* 3(2): 195-202.
[2] Hurwitz et al (2013) Evaluation of methods to concentrate and purify ocean virus communities through comparative, replicated metagenomics. *Environmental Microbiology*, 15:1428-1440.
[3] Duhaime et al (2012) Towards quantitative metagenomics of wild viruses and other ultra-low concentration DNA samples: a rigorous assessment and optimization of the linker amplification method. *Environmental Microbiology*,14:2526-37.
[4] Deng et al (2012) Contrasting life strategies of viruses that infect photo- and hetero- trophic bacteria revealed by viral-tagging. *mBio* 3(6):e00373-12. doi:10.1128/mBio.00373-12.
[5] Deng et al (2014) Viral-tagged metagenomics reveals environmental cyanophage populations are discretely clustered. *Nature*, 513, 242–245.

Presentation: Wednesday, 8 March 2017 from 9:30 - 9:45 in the Franconia Hall.

198/EEV
Fate of antibiotic resistant bacteria in two different constructed wetland types
C. Knecht*[1], M. Krüger[1,2], H. Dambeck[1], J. Nickolaus[1,2], A. Ahmed[1], S. Kellmann[1], I. Mäusezahl[1], M. Möder[3], M. Kästner[1], O. O. Adelowo[1,4], J. Nivala[5], J. A. Müller[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Environmental Biotechnology, Leipzig, Germany*
[2]*Martin-Luther-Universität Halle-Wittenberg, Institute of Biology/Microbiology, Halle, Germany*
[3]*Helmholtz Centre for Environmental Research - UFZ, Analytics, Leipzig, Germany*
[4]*University of Ibadan, Environmental Microbiology and Biotechnology, Ibadan, Nigeria*
[5]*Helmholtz Centre for Environmental Research - UFZ, Centre for Environmental Biotechnology, Leipzig, Germany*

Introduction: Wastewater treatment plants are known hotspots for the development and spreading of antibiotic resistant bacteria (ARB). Bacterial exchange of antibiotic resistance genes (ARG) might play an important role in these systems due to co-occurrence of high abundances of microorganisms and various types of environmental stress. Constructed wetlands (CWs) are considered an alternative wastewater treatment option for removal of ARB and ARG, taking advantage of the ecosystems' services that resemble those of natural wetlands. Thus, tracking ARB/ARG in CWs is of additional relevance for understanding better their fate in the environment. Here, we focused on bacterial resistance to sulfamethoxazole and trimethoprim, two antibiotics that are frequently taken in combination and are on the World Health Organization's List of Essential Medicines.
Objectives: (1) To evaluate the fate of ARB/ARG in two different types of CWs; (2) To elucidate to what extent the presence of antibiotics and other stress sources affect ARB diversity; (3) To unravel interactions of incoming bacteria with the resident/permanent microbial community of the wetlands, with main focus on horizontal gene transfer.
Materials and methods: Two pilot-scale CWs, planted with common reed and receiving the same pre-treated wastewater as inflow were investigated over a period of almost 3 years. One CW was aerated (i.e. intensified), the other was not. Abundances of sulfamethoxazole- and trimethoprim-resistant bacteria along the CWs' flow paths were quantified by plating coupled with phylogenetic identification of resistant isolates. Respective resistance genes (*sul-I*, *sul-II*, *dfrA1*) were enumerated in CW samples by qPCR analysis. The abundance of *int1* was measured as representative gene of a common mobile element carrying ARGs, i.e. class I integron. Standard wastewater parameters incl. numbers of *Escherichia coli* (quantified by MPN counting and qPCR) were recorded and bacterial community profiling was carried out via 454 pyrosequencing. Sulfamethoxazole and trimethoprim concentrations were determined by LC-MS.
Results: A significant attenuation of ARB/ARG occurred in both wetlands, the magnitude of which was larger in the aerated one. In this CW, the abundances of ARB/ARG fluctuated along the flow path. This fluctuation varied for *sul* genes and *dfrA1* and respective ARB, suggesting that stress factors may influence ARGs differently, according to the genetic context.
Conclusions: CWs are a suitable technology to attenuate ARB numbers from wastewater, with intensified systems performing better. Further analysis should be done to evaluate horizontal gene transfer within the wetlands and to which extent this is influenced by stress factors in order to optimize water treatment by the means of this inexpensive technology.

Presentation: Wednesday, 8 March 2017 from 9:45 - 10:00 in the Franconia Hall.

SHORT LECTURE 31
Microbial Cell Biology & Cellular Microbiology (SL MCB)
08 March 2017 • 08:30 – 10:00

199/MCBV
DNA- and nucleotide binding by ParC drive a capture-and-release mechanism required for cell-pole development
A. Alvarado*[1], S. Ringgaard[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Ecophysiology, Marburg, Germany*

Chemotaxis is one of the major ways bacteria sense and respond to changes in their environment and it enables motile bacteria to bias their movement towards more favorable surroundings. Chemotaxis is mediated by large multi-component clusters of signaling proteins, also known as chemotactic signaling arrays. In *Vibrio* species chemotaxis arrays are exclusively directed to one or both cell poles by a mechanism that depends on the ParA-like ATPase ParC. In new-born cells immediately after cell division, signaling arrays are localized to the old flagellated cell pole. Later in the cell cycle chemotaxis proteins are recruited to the new cell pole, resulting in a bi-polar localization pattern. Thus, at cell division each daughter cell inherits a signaling array positioned at its old flagellated pole. In the absence of ParC chemotaxis proteins are no longer properly recruited to the cell poles. Instead signaling arrays form and localize randomly along the cell length resulting in their defective inheritance upon cell division and in consequence altered motility and decreased chemotaxis. Similar to signaling arrays, ParC is localized at the flagellated old pole in recently divided cells, and is later recruited to the new pole, resulting in a bi-polar localization pattern. The cell cycle-dependent redistribution of ParC to both poles occurs by its release from the old pole and subsequent relocalization to the new pole. However, the underlying mechanism regulating polar localization of ParC and its redistribution within the cell, ultimately resulting in polar recruitment of chemotaxis protein, remains to be elucidated. Here we show that ParC non-specifically bind DNA. DNA binding is required for proper polar localization of chemotaxis arrays. Furthermore, we present data suggesting how DNA- and nucleotide binding drive a continuous cycle of capture-and-release of ParC between the cell pole and cytoplasm, which is required for ParC mediated polar recruitment of chemotaxis proteins. Importantly, the cycle likely ensures a continuous pool of cytosolic ParC available for immediate relocalization to the new pole once a ParC anchor develops at this site, hereby ensuring polar recruitment of chemotactic signaling arrays and a fully developed cell pole prior to cell division.

Presentation: Wednesday, 8 March 2017 from 8:30 - 8:45 in the Barbarossa Hall.

200/MCBV
Asymmetric DNA replication in synchronized *Vibrio cholerae*
F. Kemter*[1], S. Messerschmidt[1], N. Schallopp[1], T. Waldminghaus[1]
[1]*Philipps-Universität Marburg, SYNMIKRO, Marburg, Germany*

Vibrio cholerae, the causative agent of the cholera disease, is commonly used as model organism for bacteria with multipartite genomes. Its two chromosomes of different sizes initiate their DNA replication at different time points of the cell cycle and terminate in synchrony. To gain deeper insight into the DNA replication mechanism, we demonstrate that serine hydroxamate (SHX) based cell synchronization can be applied to *V. cholerae*. To this end, exponentially growing cells were treated with SHX, to induce the stringent response leading eventually to non-replicating cells with fully replicated chromosomes. Release of the stringent response causes the cells to initiate DNA replication in

synchrony, as determined by flow cytometry. Microarray based comparative genomic hybridization showed that DNA replication of chromosome I is initiated – as expected - before chromosome II and revealed an asymmetry in the replication of both arms of chromosome I. Thereby, the replication fork on the right replichore is around 250 kb farther away from the origin of replication than the replication fork of the left replichore. To assess conservation of DNA replication throughout the Vibrionaceae, marker frequency analyses of twelve members of this family were performed. Despite the different sizes of their secondary chromosomes, all examined strains showed the same replication behavior as *V. cholerae*: The smaller chromosome II initiates its DNA replication later than chromosome I, which leads to termination synchrony of both chromosomes. Altogether, this study contributes to the understanding of the general mechanisms governing DNA replication in bacteria with multipartite genomes.

Presentation: Wednesday, 8 March 2017 from 8:45 - 9:00 in the Barbarossa Hall.

201/MCBV

The role of phytochrome in temperature sensing

C. Streng*[1], R. Fischer[1]
[1]*Karlsruher Institut für Technologie (KIT), Institute for Applied Biosciences Department of Microbiology, Karlsruhe, Germany*

Light and temperature are two environmental signals, which can be sensed by many organisms. Microorganisms, such as filamentous fungi, can distinguish between growth in soil or at the surface through these signals. On the surface temperature shifts, osmotic stress and high amounts of reactive oxygen species pose a threat to the organism, while in the soil temperature shifts are buffered, the environment is more humid and there is less oxygen. Hence both signals are integrated in the SakA/Hog (High Osmolarity Glycerol)-Pathway, which is a general stress pathway in *Aspergillus nidulans*. Recently the integration of light via phytochrome (FphA) in the HOG-Pathway was shown [1].Interestingly, the induction of light-responsive genes was also observed after a temperature shift. The induction was reduced in the *ΔfphA* strain suggesting a role for FphA in temperature sensing. Likewise, a temperature sensing fuction was proposed recently for bacterial [2-4] and plant phytochrome [5, 6]. Fungal phytochrome resembles a bacterial two-component signaling system. The protein contains three critical amino acids, a cysteine for the covalent attachment of a linear tetrapyrrol, a histidine in the histidine-kinase domain and an aspartate in the response regulatore domain. All three amino acids were mutated alone or in combination and the ability of the protein in temperature sensing will be tested. Interestingly, the protein without the chromophore was unable to induce temperature-dependent gene induction.

References

[1] Yu, Z., O. Armant, and R. Fischer, *Fungi use the SakA (HogA) pathway for phytochrome-dependent light signalling.* Nature Microbiology, 2016. 1: p. 16019.
[2] Bai, Y., et al., *Bacteriophytochromes control conjugation in Agrobacterium fabrum.* J Photochem Photobiol B, 2016. 161: p. 192-9.
[3] Njimona, I. and T. Lamparter, *Temperature effects on Agrobacterium phytochrome Agp1.* PLoS One, 2011. 6(10): p. e25977.
[4] Njimona, I., R. Yang, and T. Lamparter, *Temperature Effects on Bacterial Phytochrome.* PLOS ONE, 2014. 9(10): p. e109794.
[5] Legris, M., et al., *Phytochrome B integrates light and temperature signals in Arabidopsis.* Science, 2016. 354(6314): p. 897-900.
[6] Jung, J.H., et al., *Phytochromes function as thermosensors in Arabidopsis.* Science, 2016. 354(6314): p. 886-889.

Presentation: Wednesday, 8 March 2017 from 9:00 - 9:15 in the Barbarossa Hall.

202/MCBV

Elucidating the composition of the outer membrane of the anammox planctomycete *Kuenenia stuttgartiensis*

M. van Teeseling*[1,2], R. Mesman[2], C. Rath[3], E. Hopmans[4], N. de Almeida[2], K. Duda[5], J. Sinninghe Damsté[4], P. Messner[3], R. Benz[6], C. Schäffer[3], M. Jetten[2], L. van Niftrik[2]
[1]*Philipps-Universität Marburg, Marburg, Germany*
[2]*Radboud University, Dept of Microbiology, Nijmegen, Netherlands*
[3]*BOKU, NanoGlycobiology Unit, Vienna, Austria*
[4]*NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry, Den Burg, Netherlands*
[5]*Research Centre Borstel, Leibniz-Center for Medicine and Biosciences, Division of Structural Biochemistry, Borstel, Germany*
[6]*Jacobs University, Department of Life Sciences and Chemistry, Bremen, Germany*

The organization of the cell envelope of *Planctomycetes* in general and anammox *Planctomycetes* in particular has been a matter of debate. Anammox bacteria have three compartments of which the innermost is a prokaryotic organelle in which the anaerobic ammonium oxidation (anammox) reaction takes place (Neumann et al, 2014). The outermost compartment has for a long time been known as the *Planctomycetes*-specific paryphoplasm (Lindsay et al, 2001), but the recent detection of peptidoglycan in this compartment suggested that this compartment should be interpreted as a periplasm (van Teeseling et al, 2015; Jeske et al, 2015). This viewpoint suggests that the outermost membrane of *Planctomycetes* is in fact an outer membrane.
Here we investigated the composition of the outer membrane in order to elucidate if OMPs and LPS are present in the outer membrane of the anammox bacterium *K. stuttgartiensis*. Therefore, purification and functional characterization of a putative OMP were performed. In addition, the presence of LPS was investigated.
This research gives the first comprehensive description of two main outer membrane components of an anammox *Planctomycete* and substantiates the emerging image of (anammox) *Planctomycetes* as Gram-negative bacteria.

References

[1] Jeske O, Schüler M, Schumann P, Schneider A, Boedeker C, Jogler M, Bollschweiler D, Rohde M, Mayer C, Engelhardt H, Spring S, Jogler C (2015) Planctomycetes do possess a peptidoglycan cell wall. Nat Commun 6: 7116.
[2] Lindsay MR, Webb RI, Strous M, Jetten MSM, Butler MK, Forde RJ, Fuerst JA (2001) Cell compartmentalisation in Planctomycetes: novel types of structural organisation for the bacterial cell. Arch Microbiol 175: 413-429.
[3] Neumann S, Wessels HJCT, Rijpstra WIC, Sinninghe Damsté JS, Kartal B, Jetten MSM, van Niftrik L (2014) Isolation and characterization of a prokaryotic cell organelle from the anammox bacterium *Kuenenia stuttgartiensis*. Mol Microbiol 94: 794-802.
[4] van Teeseling MCF, Mesman RJ, Kuru E, Espaillat A, Cava F, Brun YV, VanNieuwenhze MS, Kartal B, van Niftrik B (2015) Anammox Planctomycetes have a peptidoglycan cell wall. Nat Commun 6: 6878.

Presentation: Wednesday, 8 March 2017 from 9:15 - 9:30 in the Barbarossa Hall.

203/MCBV

Chlamydia-containing vacuole serves as deubiquitination platform to stabilize Mcl-1 and to interfere with host defense

A. Fischer*[1], K. S. Harrison[2], Y. Ramirez[3], D. Auer[1], S. R. Chowdhury[1], B. K. Prusty[1], F. Sauer[3], C. Kisker[3], P. S. Hefty[2], T. Rudel[1]
[1]*Universität Würzburg, Mikrobiologie, Würzburg, Germany*
[2]*University of Kansas, Molecular Biosciences, Lawrence, Kansas, United States*
[3]*Universität Würzburg, Rudolf Virchow Zentrum, Würzburg, Germany*

Question: *Chlamydia trachomatis* is an obligate intracellular pathogen which replicates by a biphasic life cycle within a membrane-bound vacuole called inclusion. By secretion of effector proteins, the inclusion surface serves as a signaling interface with the host cell to prevent premature host cell death and to ensure intracellular replication of *Chlamydia*. Stabilization of the anti-apoptotic protein Mcl-1 is a major mechanisms of *C. trachomatis* to block host cell death[1)]. Here, we investigate the role of the chlamydial secreted deubiquitinase Cdu1 in Mcl-1 protein stabilization and bacterial intracellular survival [2)].
Methods: By genetic manipulation of *C. trachomatis*, we generated *Chlamydia* strains expressing tagged Cdu1 or a transposon insertion mutant expressing a truncated Cdu1 protein to characterize Cdu1 expression, secretion and function. By immunofluorescence microscopy, we investigated subcellular localization of Cdu1 and Mcl-1 during infection. Furthermore, co-precipitation experiments and *in vivo* as well as *in vitro* deubiquitinase assays were used to characterize Cdu1 activity and substrate specificity. Finally, mice were challenged in an infection model.
Results: Here, we show that the chlamydial deubiquitinating enzyme Cdu1 localizes in the inclusion membrane and faces the cytosol with the active deubiquitinating enzyme domain. We identified the apoptosis regulator Mcl-1 as a target that interacts with Cdu1 and is stabilized by deubiquitination at the chlamydial inclusion. The chlamydial transposon insertion mutant in the Cdu1-encoding gene exhibited increased Mcl-1 and inclusion ubiquitination and reduced Mcl-1 stabilization. Additionally, inactivation of Cdu1 led to increased sensitivity of *Chlamydia* for IFNg and impaired infection *in vivo*.
Conclusion: Thus, the chlamydial inclusion serves as an enriched site for a deubiquitinating activity exerting a function in selective stabilization of host proteins and protection from host defense.

References

[1] Rajalingam, K., Sharma, M., Lohmann, C., Oswald, M., Thieck, O., Froelich, C. J. & Rudel, T. 2008. Mcl-1 is a key regulator of apoptosis resistance in Chlamydia trachomatis-infected cells. *PLoS One,* 3**,** e3102.
[2] Misaghi, S., Balsara, Z. R., Catic, A., Spooner, E., Ploegh, H. L. & Starnbach, M. N. 2006. Chlamydia trachomatis-derived deubiquitinating enzymes in mammalian cells during infection. *Mol Microbiol,* 61**,** 142-50

Presentation: Wednesday, 8 March 2017 from 9:30 - 9:45 in the Barbarossa Hall.

204/MCBV

Inhibition of host cell protein translation during infection with _Chlamydia trachomatis_

M. Ohmer*[1], T. Tzivelekidis[1], L. Volceanov[1], O. Schilling[2], G. Häcker[1]
[1]*University Medical Center, Department of Medical Microbiology and Hygiene, Freiburg, Germany*
[2] *Institut für Molekulare Medizin und Zellforschung, Zentrum für Biochemie und Molekulare Zellforschung (ZBMZ) , Freiburg, Germany*

Chlamydia trachomatis is an obligate intracellular pathogen that is the recognized as the leading cause of bacterial sexually transmitted disease. It infects the epithelium of the urethra of men and endocervix of woman, but is additionally associated with blindness of millions of people worldwide as well as pelvic inflammatory disease. *Chlamydia trachomatis* has an intracellular biphasic developmental cycle: after uptake of the host cell *Chlamydia* develops in a membranous vacuole in the cytoplasm, a so called inclusion, that protects it from host defenses and phagolysosomal degradation. Due to its intracellular life style, numerous changes in the host cell functions such as cell division, organelle structure, immune signaling and apoptosis are relevant to chlamydial development. These effects are believed to be achieved by effector proteins that are secreted through the type III-secretion system into the inclusion membrane or the cytosol of the host cell. However, not many of those proteins have been identified that are clearly linked to an observed effect.
We investigated the effect of infection on host cell protein synthesis and established a proteomic map of degradation and synthesis of host cell proteins during chlamydial infection. SILAC-labeling of either uninfected or infected HeLa cells showed a considerable reduction of host translation associated proteins. Additionally, a substantial reduction of *de novo*-protein synthesis was seen in HeLa cells infected with *C. trachomatis* from about 24 h post-infection. Ribosomal profiling showed a large increase in the 80S ribosome population and a reduction in the polysomal fractions during infection. Analysis of polysome-bound (actively translating) mRNAs showed the translational down-regulation of a number of host pathways. Thus, *C. trachomatis* reduces protein synthesis in the infected cells, very likely through targeting of ribosomal protein translation. This may be relevant to counter a host response to infection and may explain numerous cell-biological effects observed during chlamydial infection.

Presentation: Wednesday, 8 March 2017 from 9:45 - 10:00 in the Barbarossa Hall.

SHORT LECTURE 32
Metabolic Engineering - From Fluxes to Products (SL SMB)
08 March 2017 • 08:30 – 10:00

205/SMBV

Production of Polyhydroxyalkanoates in Defined Mixed Cultures from CO_2 and Light

H. Löwe[1], K. Pflüger-Grau*[1], A. Kremling[1]
[1]*Technische Universität München, Systems Biotechnology, Garching b. München, Germany*

Pollution of the ocean with plastics at a rate of up to 12.7 million tons per year of is a severe threat to the aquatic ecosystem [1]. One solution to this problem is the use of biodegradable plastics, like polyhydroxyalkanoates (PHAs) that are slowly decomposed in natural ecosystems. Unfortunately, these compounds are still more expensive than plastics made from fossil resources, especially due to the price of the substrates used for their production.
To address this hurdle, we studied the production of PHA in a defined mixed culture between *Synechococcus elongatus* PCC7942 *cscB* and *Pseudomonas putida*. *S. elongatus cscB* is able to produce sucrose from CO_2 and light under salt stress [2], which potentially could reduce the substrate costs on the long run considering the lower productivity and the additional process steps for crop-derived substrates. *P. putida* KT2440 is a natural PHA producer, but is not able to metabolize sucrose. Therefore, we modified this organism by introducing heterologous genes for sucrose metabolism.
Both strains were cultivated in the a lab-scale photobioreactor at the same time under nitrogen-limiting conditions, and PHA production, growth and sucrose concentration were measured by GC, flowcytometry and HPLC.

In a period of three weeks, *S. elongatus* grew linearly and reached a sucrose concentration of about 2.7 g/L while *P. putida* consumed sucrose and grew to about 10% of the cell count of *S. elongatus*. Due to the nitrogen-limition, *P. putida* started to produce significant amounts of PHA one week after inoculation, as monitored by nile red staining and GC. Growth of *P. putida* was limited only by the ability to split sucrose – a problem that will be tackled by constructing more efficient sucrose utilizing strains.
This process illustrates the potential of synthetic mixed cultures for the production of chemicals from ubiquitous and cheap resources. By co-cultivating both strains, the risk of contamination of the sucrose producing cyanobacteria is reduced. The sugar substrate sucrose does not have to be grown, extracted and transported such as when using conventional crops, but is readily available in the photobioreactor. Given further optimization, this process could be a viable alternative for bioplastics and chemical production in the future.

References
[1] Jambeck *et al.* (2015) *Science.* 347: 768–771
[2] Ducat *et al.* (2012) Appl. Environ. Microb. 78(8): 2660-2668

Presentation: Wednesday, 8 March 2017 from 8:30 - 8:45 in room 13.

206/SMBV
Fixing the Calvin Cycle: CO_2 Fixation Through an Artificial Photorespiratory Bypass Build From Non-natural Reactions
M. Scheffen*[1], J. Zarzycki[1], A. Bar-Even[2], T. J. Erb[1]
[1]*MPI for Terrestrial Microbiology, AG Erb, Marburg, Germany*
[2]*MPI of Molecular Plant Physiology, Potsdam, Germany*

Photoautotrophic organisms, including plants and thus agricultural productivity, are dependent on the performance of CO2 fixation in the Calvin cycle. Its key enzyme, Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), is a quite inefficient carboxylase, because the enzyme cannot discriminate between CO2 and O2. If the oxygenase side reaction takes place, the toxic intermediate 2-phosphoglycolate is formed and has to be detoxified in an energy-demanding and CO2 releasing process called photorespiration.
In this project, we aim at creating more efficient, synthetic photorespiration bypasses based on rational design. Such synthetic pathways should consider several aspects:
(i) The individual reaction steps should not release CO2.
(ii) The energy demand in form of ATP and reducing equivalents should be minimal.
(iii) The reaction sequence should be short and involve as few enzymes as possible.
We propose a tartronyl-CoA bypass that is based on the conversion of glycolate to glycolyl-CoA, followed by its carboxylation to tartronyl-CoA and two subsequent reduction steps to yield glycerate, which can be phosphorylated to 3-phosphoglycerate replenishing the Calvin cycle.
By searching databases and literature, we identified candidate enzymes that could be able to catalyze the required reactions for the tartronyl-CoA bypass. We focused on enzymes that can perform analogous reactions with structurally similar substrates. We then biochemically characterized and, if needed, further engineered and/or evolved these candidate enzymes towards higher catalytic efficiencies. Enzymatic activities were determined spectrophotometrically and by high performance liquid chromatography coupled to high resolution mass spectrometry (HPLC-hrMS).
We demonstrated that all reactions of the photorespiratory tartronyl-CoA are possible and biochemically characterized all required enzymes. The activation of glycolate to glycolyl-CoA can be performed by a CoA transferase or a synthetase. The subsequent carboxylation reaction is the most challenging one of the photorespiratory bypass, because such a reaction is not known to exist in any natural metabolic pathway. We created a mutant of a biotin dependent carboxylase that is able to carboxylate glycolyl-CoA to tartronyl-CoA and were able to increase its specific activity more than two orders of magnitude. We have identified a bi-functional enzyme that is able to catalyze the final two reduction steps, yielding glycerate.
The proposed synthetic metabolic pathway has great potential to be implemented in photoautotrophic organisms in the future to enhance photosynthetic efficiency, because it requires less enzymes and less energy than the natural photorespiration pathway and, as its greatest advantage, fixes CO2 instead of releasing it.

Presentation: Wednesday, 8 March 2017 from 8:45 - 9:00 in room 13.

207/SMBV
Treatment of industrial wastewater and energy generation via Microbial Fuel Cells (MFC)
S. Epple*[1], J. Gescher[1]
[1]*KIT Karlsruhe, Institute of Applied Sciences, Karlsruhe, Germany*

Over the past decades, the demand of freshwater increased immensely. Besides freshwater scarcity, environmental pollution and fossil fuel depletion are important environmental challenges. With regard to these facts Microbial Fuel Cell (MFC) – technology gained much attention in the recent years in view of a sustainable and environmental friendly method for wastewater treatment and current production. Wastewater contains a high amount of energy in the form of organic matter which is normally not used. In contrary, the elimination of this organic carbon is the most energy demanding step in wastewater treatment. The application of MFC-technology for wastewater treatment would overcome this problem by a direct conversion of chemical into electrical energy. The aim of this study was to decrease the high amount of organic acids in a real industrial wastewater stream using a bioelectrochemical system. In a first step exoelectrogenic microorganisms were isolated from this particular industrial wastewater. In a second step it was tested whether these isolates or the isolates and an exoelectrogenic model organism were able to eliminate the organic carbon load from the wastewater. The experiments were conducted first under batch-mode conditions for 21 days. In parallel the same experiment was conducted under flow through conditions for 94 days with different hydraulic retention times (HRT). The comparison of the two inoculation regimes under the two conditions (batch-system and flow-through system) resulted in a better performance of the setup containing also the exoelectrogenic model organism. The highest average current density (1200 mA/m2) was measured in the flow-through system of the setup inoculated with the isolates and the model organism. The similarly inoculated batch-system produced only a current of 15 mA/m2, which is 80-fold less than in the flow-through system. Inoculation with new isolates only lead under flow-through conditions to an average current density of 600 mA/m2 compared to 7.6 mA/m2 under batch conditions (78-fold less). The TOC (total organic carbon) removal mirrored the results from current measurements and was highest under flow through conditions with the mixture of the novel isolates and the exoelectrogenic model organism (75% TOC removal). According to our results, a flow-through system and the addition of a exoelectrogenic model organism members the natural wastewater community was more efficient for treating this type of wastewater than a batch-system.

Presentation: Wednesday, 8 March 2017 from 9:00 - 9:15 in room 13.

208/SMBV

Metabolic engineering of *Escherichia coli* for biosynthesis of 2-(4-Aminophenyl)ethanol (PAPE)

B. Mohammadi Nargesi*[1], G. A. Sprenger[1], J. W. Youn[1]
[1]*Institute of Microbiology, Stuttgart, Germany*

Introduction: 2-(4-Aminophenyl)ethanol (PAPE) is an important side chain building block which is widely used for the synthesis of aromatic polyamides [1], polymers [2] and Myrbetriq® which is used for overactive bladder treatment (OAB) [3]. Although the chemical synthesis of PAPE has been achieved [4], chemical synthesis processes are often not environmentally friendly, so microbial biosynthesis of PAPE would be an alternative.
Objectives: We report a novel approach for biosynthesis of PAPE from glucose via genetic modification of the shikimate pathway (via aminodeoxychorismate (ADC)) in *E. coli*. In this study, we intend to construct an efficient biosynthesis pathway for the production of PAPE in *E.coli* from glucose.
Methods: To construct a PAPE synthetic pathway, the genes *pabA*B (ADC synthase) from *Corynebacterium glutamicum, papB* (ADC mutase), *papC* (ADC dehydrogenase) from *Streptomyces venezuelae* and *aro10* (encoding phenylpyruvate decarboxylase) from *Saccharomyces cerevisiae* were heterologously expressed in *E. coli FUS4* [5] in shake-flask cultivations and fed batch. In addition, three genes from *E.coli* which encodes 3-deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthase (*aroF*), 3-dehydroquinate synthase (*aroB*), and shikimate kinase II (*aroL*) were cloned and co-overexpressed with four required genes for PAPE production in strain *E.coli FUS4*. The recombinant *E. coli* strains were cultured in shake-flasks with fed-batch and investigated for the PAPE production by HPLC and LC-MS.
Results: The introduction and expression of four genes (*pabA*B, *papB*, *papC & aro10*) for PAPE synthesis into *E. coli FUS4* led to a titer of 0.12g/l PAPE from 5g/l glucose. By co-expression of three genes involved in shikimate pathway (*aroF, aroB*, *aroL)* the flux towards chorismic acid (a main precursor for production of PAPE) was enhanced and finally a PAPE titer of 0.25 g/l from 5g/l glucose within 48 h was reached. By optimization of growth conditions including increase concentration of tyrosine, phenylalanine and glucose fed-batch, a PAPE titer of about 1.8 g/l was achieved.
Conclusions: We demonstrate that PAPE can be produced with good yield and titer by an engineered *Escherichia coli* from glucose.

References

[1] Masuo S, Zhou S, Kaneko T, Takaya N. *Scientific Reports*. 2016; 6,25764.
[2] Li L, Yonetake K, Haba O, Endo T, Ueda M. *Polymer J.*2001; 33(4), 364-70.
[2] Sacco E, Bientinesi R. *Therapeutic advance in urology*.2012; 4(6): 315–324.
[4] Schul" tsev AL. *Russian journal of general chemistry*. 2011; 81(11) 1849–1852.
[5] Gottlieb K, Albermann C, Sprenger GA. *Microb Cell Fact*. 2014; 13:96.

Presentation: Wednesday, 8 March 2017 from 9:15 - 9:30 in room 13.

209/SMBV

Exploiting unconventional secretion for production of biopharmaceuticals in *Ustilago maydis*

M. Terfrüchte*[1], S. Wewetzer[2], J. Büchs[2], M. Feldbrügge[1], K. Schipper[1]
[1]*Heinrich Heine University Düsseldorf, Institute for Microbiology, Düsseldorf, Germany*
[2]*RWTH Aachen, Department of Biochemical Engineering, Aachen, Germany*

Biotechnological production of pharmaceuticals, natural compounds and chemicals is gaining more and more importance in the healthcare industry. The development and production of recombinant proteinaceous medicals is thus of big interest.
Every protein individually has highly specific demands on the expression host, for example with regard to posttranslational modifications such as *N*-glycosylation or disulfide bonds. Hence, a broad variety of expression hosts is required. A recent member of these expression hosts is the basidiomycete *Ustilago maydis*. Former studies revealed that this fungus uses an unconventional secretion pathway which circumvents *N*-glycosylation to export the endochitinase Cts1. To exploit this feature for the production of heterologous proteins in *U. maydis*, the protein of interest is fused to the Cts1 protein by an immuno-detectable linker and tagged with various purification tags.
This study is focusing on the optimization of expression and characterization of camelid derived nanobodies as a proof of principle for the production of biopharmaceutically relevant targets. The general optimization of media composition and conditions for large scale cultivations is conducted in parallel in order to establish *U. maydis* as an industrially relevant expression host. To this end, application of RAMOS (Respiration Activity MOnitoring System) devices in combination with offline analytics enabled detailed cultivation studies allowing fast identification and elimination of limiting factors. Optimized conditions will later be applied on and adjusted to bioreactor cultivations with the overall goal to increase product yields.

Presentation: Wednesday, 8 March 2017 from 9:30 - 9:45 in room 13.

210/SMBV

Opportunities and challenges of synthesis gas utilization by the facultative chemolithoautotrophic bacterium *Ralstonia eutropha* H16

D. Heinrich*[1], C. Klask[1], M. Raberg[1], A. Steinbüchel[1]
[1]*Westfälische Wilhelms-Universität, IMMB, Münster, Germany*

As a common inhabitant of soil and freshwater, the β-proteobacterium *R. eutropha* H16 is able to adapt to fluctuating levels of oxygen by switching from aerobic to anaerobic respiration (Aragno & Schlegel, 1992). Additionally, *R. eutropha* can fix CO_2 via the Calvin-Benson-Bassham (CBB) cycle and utilize hydrogen with the help of two energy-conserving hydrogenases. Since *R. eutropha* can be cultivated to high cell densities, accumulates large amounts of poly(3-hydroxybutyrate) and is amenable to genetic modification, it harbors promising commercial potential.
Industrial waste gases and gases generated from gasification or pyrolysis of organic residues, commonly referred to as synthesis gas (syngas), present favorable feedstocks to sustainably produce so-called second generation bioproducts. Syngas contains primarily CO, H_2 and CO_2. Although *Ralstonia eutropha* H16´s genome harbors eight putative clusters that comprise *cox*-genes, which presumably code for a carbon monoxide dehydrogenase (CODH), the strain cannot utilize CO as the sole source of carbon and energy like it has been shown for other bacteria synthesizing a functional CODH. However, the bacterium is principally able to grow with syngas by metabolizing the present CO_2 and H_2. In this study the utilization of syngas by *R. eutropha* wild type and recombinant strains was examined. *R. eutropha* showed

considerable tolerance towards carbon monoxide when cultivated in atmospheres containing up to 20% (vol/vol) CO, which did not affect growth significantly. Aside from investigating a possible role of *R. eutropha´s* own *cox*-genes, it was shown that heterologous expression of genes coding for CO-dehydrogenases of the facultative anaerobic purple bacterium *Rhodospirillum rubrum* S1 or the aerobic *O. carboxidovorans* OM5 led to increased growth with syngas when cultivated under the respective conditions. Moreover, anaerobic syngas cultivations at denitrifying conditions were conducted and improved to take advantage of the energy efficient anaerobic respiration and to avoid explosive knallgas that occurs from mixtures of O_2 and H_2. This study demonstrates the possibility of (i) producing different biopolymers as well as fine chemicals from syngas with genetically engineered strains of *R. eutropha*, while (ii) concomitantly removing harmful compounds such as CO_2 and NO_3^- from the environment.

References

[1] Aragno, M. & Schlegel, HG (1992) The mesophilic hydrogen-oxidizing (knallgas) bacteria. In *The Prokaryotes* (eds. Ballows, A., Trüper HG, Dworkin, M., Harder, W., & Schleifer, K.H.) 344-385 (Springer-Verlag, New York, NY).

Presentation: Wednesday, 8 March 2017 from 9:45 - 10:00 in room 13.

SHORT LECTURE 33
Phage defense and therapeutics (SL PC)
08 March 2017 • 08:30 – 10:00

211/PCV
Engineering the broad-host P1 bacteriophage for the delivery of programmable-spectrum CRISPR antimicrobials

C. Beisel*[1]
[1]*North Carolina State University, Chemical and Biomolecular Engineering, Raleigh, North Carolina, United States*

The growing instances of multidrug-resistant infections and the importance of the human microbiome call for targeted antimicrobial agents that circumvent antibiotic resistance and can be programmed to selectively eliminate some bacteria but not others. One promising avenue comes from CRISPR-Cas immune systems in bacteria and archaea. These systems rely on guide RNAs to direct the systems Cas proteins to cleave complementary foreign genetic material. By introducing synthetic guide RNAs that target the bacterial genome, we previously showed that these same systems could be tricked into introducing a lethal double-stranded genomic break, resulting in programmable and sequence-specific killing. Because this mechanism does not rely on small-molecule antibiotics, CRISPR antimicrobials would be effective against multidrug resistant pathogens.

With this demonstration, the next major challenge is delivery. Bacteriophages have proven to be promising delivery vehicles based on their ability to inject their genetic material into the cytoplasm and the multitude of bacteriophages found in nature. Bacteriophages have also been used to deliver CRISPR-Cas systems to clear or prevent the dissemination of antibiotic resistance plasmids. Despite these advances, the vast majority of bacteriophages are species-specific and even strain-specific, severely restricting which bacteria can be targeted with CRISPR antimicrobials. This raised the question: how can bacteriophages be used to deliver CRISPR antimicrobials to a broad range of bacteria?

Here, we engineered the broad-host temperate bacteriophage P1 for the efficient delivery of CRISPR antimicrobials across taxa. P1 is known to inject its DNA into diverse bacteria that fall within the Proteobacterium phylum, and phagemids—plasmids with phage packaging sequences—have been developed for the delivery of synthetic DNA. We found that the P1 phagemid is packaged and delivered 100-fold less frequently than the phage genome, leading us to encode CRISPR antimicrobials in the P1 genome. We identified multiple landing sites within the genome that can accommodate synthetic DNA, and inserting DNA into these sites improved P1 as a delivery vehicle by preventing spurious phage production and allowing multiple rounds of infection. Finally, we found that the P1 genome equipped with an entire CRISPR-Cas could be delivered to bacteria across the Proteobacterium phylum and could elicit programmable-spectrum killing. These findings highlight the promise of broad-host bacteriophages for the delivery of CRISPR antimicrobials and their use to combat multidrug resistant pathogens and rationally shape the human microbiome.

Presentation: Wednesday, 8 March 2017 from 8:30 - 8:45 in room 10-11.

212/PCV
Acquisition of the bacterial trancription machinery by phage-encoded factors

R. Wigneshweraraj*[1]
[1]*Imperial College , MRC Centre for Molecular Bacteriology, London, United Kingdom*

The bacterial transcription machinery, the RNA polymerase (RNAP), represents a nexus for the regulation of bacterial gene expression. Unsurprisingly, phages, viruses that infect bacteria, have evolved diverse and elegant strategies to alter the activity of bacterial (host) RNAP during infection to shift host resources towards the production of viral progeny. Therefore, during phage infection, the bacterial RNAP can also be subjected to regulation by phage-encoded regulatory factors. The molecular basis by which phage-encoded regulatory factors modulate the bacterial RNAP is poorly understood, and in this presentation I will describe the mechanism by which two small T7 proteins modulate the host RNAP at different stages of the infection process to ensure optimal phage progeny production. I will also describe how the results have contributed to advance our understanding of adaptive transcriptional responses in bacteria and insipre and inform new antibiotic discovery.

Presentation: Wednesday, 8 March 2017 from 8:45 - 9:00 in room 10-11.

213/PCV
Dynamic biofilm architecture confers individual and collective mechanisms of phage protection

L. Vidakovic*[1], P. Singh[1], R. Hartmann[1], C. Nadell[1], K. Drescher[1,2]
[1]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[2]*Philipps University Marburg, Department of Physics, Marburg, Germany*

In Nature, bacteria live in surface attached communities, termed biofilms, which are found in every habitat where bacteria are present [1]. Within these communities bacteria secrete an extracellular matrix that can protect cells from exogenous stress [2]. In the environment biofilms may encounter the presence of viral pathogens termed bacteriophages (or simply: phages) which use bacteria as their host for self-replication [3]. In order to understand the interaction of phages and biofilms, as well as the survival of these communities during phage attack, we developed a method to visualize phage spread inside living *E. coli* AR3110 biofilms. By insertion of *sfgfp* into the T7 phage genome, the conversion of susceptible to infected cells can be monitored in space and time using a confocal microscope. We discovered that biofilm susceptibility to phage infection was dependent on the stage of biofilm development. Biofilms that were grown for 48 h or less were rapidly eradicated due to phage infection. By contrast, biofilms grown for 60 h and more experienced no

biomass reduction during phage exposure. In order to understand the mechanism by which biofilms can endure phage attack, we examined the potential role of the biofilm matrix in the development of phage tolerance. The removal of curli, a major component of the *E. coli* matrix [4], generated biofilms that were rapidly infected by phages, regardless of their biofilm age. Visualization of curli fibers via immunostaining within growing biofilms further demonstrated a dynamic change in matrix composition. Production of curli fibers starts between 48 h and 60 h of biofilm growth corresponding exactly to the time when biofilms become tolerant to phage exposure. We further discovered that curli-dependent biofilm protection is achieved by two mechanisms: (1) Curli prevent phages from diffusion inside biofilms, and (2) curli fibers protect individual cells from phage infection. Our results show that a single component of the biofilm matrix can provide individual as well as collective protection against phage infection.

References

[1] H.-C. Flemming *et al.*, *Nature Reviews Microbiology*, 2016, 14, 563.
[2] H.-C. Flemming, J. Wingender, *Nature reviews. Microbiology*, 2010, 8, 623.
[3] J.E. Samson *et al.*, *Nature reviews. Microbiology*, 2013, 11, 675.
[4] L. Hobley *et al.*, *FEMS Microbiology Reviews*, 2015, fuv015.

Presentation: Wednesday, 8 March 2017 from 9:00 - 9:15 in room 10-11.

214/PCV

Enzymatic Promiscuity and Specificity Determinants of a CRISPR-Cas6 RNA Endonuclease

W. Hess*[1], R. Jesser[1], J. Behler[1], C. Benda[2], S. Lott[1]
[1]*University of Freiburg, Genetics & Experimental Bioinformatics, Freiburg, Germany*
[2]*Max Planck Institute of Biochemistry, Department of Structural Cell Biology, Martinsried, Germany*

Question: A hallmark of CRISPR-Cas defense mechanisms are the crRNAs that guide associated proteins in the destruction of invading DNA or RNA. Therefore, specialized RNA endonucleases are critical for efficient activity of the CRISPR-Cas defense mechanisms. Cas6-type enzymes are the maturation RNA endonucleases in many Type I and Type III CRISPR-Cas systems. These enzymes are of amazing sequence diversity. Therefore, the critical residues involved in the recognition and cleavage of RNA substrates and the substrates themselves need to be addressed. In the cyanobacterium *Synechocystis* sp. PCC 6803 three separate CRISPR-Cas systems are present (1). Based on genetic, transcriptomic and biochemical evidence, two associated endoribonucleases, Cas6-1 and Cas6-2a, were determined to be involved in crRNA maturation from two of these systems (2).
Results: We found that despite a sequence identity of less than 20%, Cas6-1 and Cas6-2a exhibit substrate promiscuity *in vitro*, to process not only their cognate transcript substrates, but also the respective non-cognate precursors, whereas they are specific *in vivo*. Therefore, we systematically mutated both the substrate repeats of Cas6-1 as well as the enzyme at multiple conserved residues supposed to be involved in RNA binding or catalytic activity. The mutation of conserved amino acids revealed R29, H32-S33 and H51 as absolutely essential, whereas the mutation of R175A-R176A had a detrimental effect on the enzyme activity and the K155A mutation led to a slight reduction in enzymatic activity. In contrast, the mutations R67A, R81A and K231A left the enzymatic activity unchanged. These results are consistent with the predominant role of histidines in the active site and of positively charged residues in RNA-binding. The systematic variation of the substrate repeat revealed a surprisingly high tolerance towards the length of the repeat hairpin and 5' handle and several multiple nucleotide positions. Taking this information into account we scanned genome-wide for the inferred RNA motifs and characteristic signals in the transcriptome, revealing possible cleavage sites in several transcripts of genes unrelated to the CRISPR-Cas system.
Conclusions: The protein-RNA interaction site appeared to differ from other known systems, illuminating the phantastic plasticity of native CRISPR-Cas systems. Cas6 cleavage of host transcripts would add another facette to the growing number of examples for the involvement of CRISPR-Cas systems in functions unrelated to defense.

References

[1] Scholz I., Lange S.J., Hein, S., Hess W.R., Backofen R. (2013) CRISPR-Cas systems in the cyanobacterium Synechocystis sp. PCC6803 exhibit distinct processing pathways involving at least two Cas6 and a Cmr2 protein. PLoS ONE, 8(2), e56470.1-e56470.15
[2] Reimann V., Alkhnbashi O., Saunders S.J., Scholz I., Hein S., Backofen R., Hess W.R. (2016) Structural constraints and enzymatic promiscuity in the Cas6-dependent generation of crRNAs. Nucleic Acids Research, *Epub ahead of print*. DOI:10.1093/nar/gkw786

Presentation: Wednesday, 8 March 2017 from 9:15 - 9:30 in room 10-11.

215/PCV

Phage application aiming at the reduction of ESBL-producing *E. coli* in livestock farming

I. Schmidt*[1], A. Bierbrodt[2], J. Wittmann[1], S. Kittler[3], R. Mengden[3], T. Lehnherr[4], M. Fink[2,4], G. Klein[3], C. Rohde[1], M. Rohde[5], H. Lehnherr[4]
[1]*Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[2]*FINK TEC GmbH, Hamm, Germany*
[3]*University of Veterinary Medicine Hannover, Institute for Food Quality and Food Safety, Hannover, Germany*
[4]*PTC Phage Technology Center GmbH, Bönen, Germany*
[5]*Helmholtz Centre for Infection Research, Central Facility for Microscopy, Braunschweig, Germany*

Introduction: The development of new agents to control pathogens is urgently needed in an era which is characterized by the presence of multidrug resistance (MDR) in bacteria. Not only the spread of MDR in the clinical area, livestock farming and food processing but also the impact on the environment is worrying from a public health perspective.
Together with various forms of *Escherichia coli* infections, colibacillosis is the most common bacterial disease of poultry and causes significant economic losses. Standard therapies include the use of antibiotics which promotes the selection of multidrug-resistant ESBL (extended-spectrum β-lactamase)-producing *E. coli*. As a zoonotic pathogen, *E. coli* can be transmitted to humans via the food chain, posing a serious health risk.
Phages are bacterial viruses that can infect and lyse only strains of the same bacterial species without affecting other bacteria in the environment, making them attractive for application in medicine and food production. Based on current knowledge pure preparations of phages are considered to be safe with no side effects recorded.
Objectives: In accordance with the One-Health concept that was a leading theme during the recent World Health Summit, this project aims to isolate and characterize phages for the use against ESBL *E. coli* infections in broilers and to test the *in vivo* efficacy of a phage preparation to reduce the application of antibiotics in livestock farming.
Material & Methods: Phages were isolated from environmental samples collected from various places in Germany and host range analysis was achieved using standard methods. Phages with a broad host range were further characterized by TEM and

bioinformatic analyses of phage genomes. Lysis kinetics were performed to study the *in vitro* efficiency. For the identification of a trackable strain in animal experiments, we performed antibiograms of 10 ESBL-producing *E. coli* isolates from poultry skin.

Results: Thus far, we isolated phages from sewage (21), surface water (4), manure (34), poultry (18) and horse dung (1) and hospital wastewater (5). The characterization revealed a diversity of morphotypes (77% *Myo*-, 6% *Podo*-, and 17% *Siphoviridae*) and similarities in particular to T4- or T5-like phages.

We identified E28 as suitable strain for tracing *in vivo* due to its rare kanamycin resistance. So far, we found 11 phages that infect E28 with varying efficiency. In order to avoid the development of phage resistance we composed a cocktail of 6 phages, which differ in host coverage, genotype and inhibit the culture growth.

Conclusion: The origin of phages has no impact on the host range. Phages do not distinguish between ESBL-producing and sensitive strains and enable us to reduce the selective pressure for MDR and the presence of zoonotic bacteria at the same time. Because of their specific bactericidal activity, phages have the potential to be used as a sustainable One-Health approach.

Presentation: Wednesday, 8 March 2017 from 9:30 - 9:45 in room 10-11.

216/PCV

CRISPRi as a tool to repress gene expression: Analysis of the tRNA splicing endonuclease of *Haloferax volcanii*

T. S. Schwarz*[1], S. J. Berkemer[2], C. J. Daniels[3], P. F. Stadler[2], A. Marchfelder[1]
[1]*Ulm University, Bio II, Ulm, Germany*
[2]*Leipzig University, Bioinformatics, Leipzig, Germany*
[3]*The Ohio State University, Microbiology, Columbus, Ohio, United States*

So far no tools are available for gene repression in archaea, which makes *in vivo* studies on essential genes difficult. This can now be resolved using CRISPRi, a method for programmed gene repression that was established within the last few years. CRISPRi, short for CRISPR interference, harnesses the CRISPR-Cas system to repress the expression of a specific gene *in vivo*. It has been successfully employed to down regulate genes in bacteria as well as in eukaryotes (1). Just recently we established CRISPRi for archaea in the model organism *H. volcanii* using the endogenous CRISPR-Cas type I-B (2).

Using CRISPRi we successfully repressed transcription of the essential gene for the splicing endonuclease *endA*, which resulted in reduction of the *endA* transcript to 40 % compared to the wildtype strain. The splicing endonuclease is one of the proteins involved in tRNA maturation, since it removes the introns from tRNA precursors. The enzyme recognizes and cleaves the bulge-helix-bulge (BHB) motif present in the introns of tRNAs (3, 4). In CRISPRi cells, unspliced $tRNA^{Trp}$ precursors accumulated and a reduction of mature $tRNA^{Trp}$ levels was observed. Furthermore we observed effects on the maturation of the 16S rRNA and severe growth defects compared to the control strain.

To investigate the substrate specificity of the tRNA splicing endonuclease we expressed it as recombinant protein and successfully carried out *in vitro* processing experiments using $tRNA^{Trp}$, a known substrate, and a potential substrate, the 16S rRNA. Additional potential substrates of the splicing endonuclease with a BHB motif will be identified by bioinformatics analysis. The identified substrates will be tested using the established *in vitro* processing assay. In addition, we will verify the new substrates by comparing their processing in wild type and CRISPRi cells, that repress the splicing endonuclease expression. We expect unprocessed substrates to accumulate in CRISPRi cells.

References

[1] Luo M.L, Leenay R.T, Beisel C.L. Current and Future Prospects for CRISPR-Based Tools in Bacteria. Biotechnol. Bioeng., 2016, vol. 113, no. 5, pp. 930-943.
[2] Stachler A-E; Marchfeler A. Gene Repression in Haloarchaea using the CRISPR (clustered regularly interspaced short palindromic repeats) - Cas I-B system. JBC, 2016, vol.291, no. 29, pp. 15226-15242.
[3] Thompson L. D; Daniels C. J. Recognition of Exon-Intron Boundaries by the Halobacterium volcanii tRNA Intron Endonuclease. JBC, 1990, vol. 265, no. 30, pp. 18104-12111.
[4] Chant, J. and Dennis, P. Archaebacteria: Transcription and processing of ribosomal RNA sequences in Halobacterium cutirubrum. EMBO J., 1986, vol. 5, no. 5, pp. 1091-1097.

Presentation: Wednesday, 8 March 2017 from 9:45 - 10:00 in room 10-11.

SHORT LECTURE 34
Archaea (SL AR)
08 March 2017 • 08:30 – 10:00

217/ARV

Structure of the methanogenic heterodisulfide-reductase/hydrogenase complex catalysing ferredoxin reduction using electron-bifurcating mechanism.

T. Wagner*[1], J. Koch[1], U. Ermler[2], S. Shima[1]
[1]*Max Planck Institute for terrestrial microbiology, Biochemistry, Marburg, Germany*
[2]*Max Planck Institute for biophysics, Frankfurt, Germany*

Methanogenic archaea are able to produce methane, a potent greenhouse gas considered to contribute to global climate changes (1). The methanogenic pathway constitutes their source of energy, with approximately half of an ATP per formed methane. To save ATP, the hydrogenotrophic methanogens developed a unique CO2 fixation enzyme, the formylmethanofuran dehydrogenase, which does not require ATP hydrolysis for the CO2-fixation reaction (2). However, formylmethanofuran dehydrogenase requires low-potential electron donor such as reduced ferredoxin. The heterodisulfide-reductase:hydrogenase complex (HdrABC-MvhAGD) reduces ferredoxin by electron bifurcation mechanism using H2 as electron donor and heterodisulfide of coenzyme M and coenzyme B (CoM-S-S-CoB) as the high potential electron acceptor (3). CoM-S-S-CoB is produced during the methane forming reaction, which is the last step of the methanogenesis.

The homologues of HdrA are found in numerous metabolisms in archaea and bacteria (sulfur reduction, acetogenesis, etc...). Moreover, HdrB contains the CCG motif, which is possibly involved in the heterodisulfide reductase reaction and is present in more than 2000 other proteins (4). Therefore, it is of importance to elucidate the structure of the complex at the atomic scale.

We natively purified and crystallized HdrABC-MvhAGD from a hydrogenotrophic methanogen under anoxic environment. The anomalous signal from the intrinsic irons helped us to solve the structure by X-ray crystallography at a resolution of 2.3 Å.

The structure indicated that the HdrABC-MvhAGD complex is organized as a core of HdrA dimer surrounded by the peripheral catalytic units: the [NiFe]-hydrogenase MvhAG and the heterodisulfide reductase center HdrBC. MvhD is a relay to transfer the electrons from the hydrogenase to the flavin bound on HdrA.

Unexpectedly, HdrA contains two extra [4Fe-4S] clusters, which cannot be predicted because of the unique coordination motif. These [4Fe-4S] clusters could be involved in the electron bifurcation. The two CCG motif contained in HdrB coordinate two pentacoordinated non-cubane [4Fe-4S] clusters at the heterodisulfide reductase active site. The freeze trapping of the reaction intermediates, from CoM-S-S-CoB allowed us to propose

a catalytic mechanism. The disulfide bond is cleaved at both non-cubane [4Fe-4S]-clusters and formed a covalent bond with an iron from each non-cubane cluster.
The HdrABC structure will serve as a prototype for multiple homologues because HdrA appears to be an universal module for electron bifurcation and HdrB as [4Fe-4S]/disulfide-bond switch apparently applied in diverse metabolic pathway by numerous organisms.

References
[1] Thauer *et al.*, Nat. Rev. Microbiol. 6, 579-591 (2008)
[2] Wagner *et al.,* Science 354,114-117 (2016)
[3] Kaster *et al*., Proc. Natl. Acad. Sci. USA 109, 15084–15085 (2012)
[4] N. Hamann *et al.*, Biochemistry 46, 12875 (2007)

Presentation: Wednesday, 8 March 2017 from 8:30 - 8:45 in room 5.

218/ARV
Archaeal ultrastructure and motility: the case of *Pyrococcus furiosus*
A. Bellack*[1], F. Heiss[1], R. Reichelt[1], V. Heinz[1], R. Wirth[1], B. Daum[2,3], R. Rachel[1]
[1]*University of Regensburg, Microbiology, Regensburg, Germany*
[2]*MPI biophysics, Structural Biology, Frankfurt, Germany*
[3]*University of Exeter, Living Systems Institute, Exeter, United Kingdom*

Introduction: *Pyrococcus furiosus* is an organotrophic, hyperthermophilic euryarchaeum, which serves as a model system in archaeal molecular cell biology. Cells are motile by means of about 50 flagella, with an optimum at 95° to 100°C. Motility is directly observed using a phase contrast light microscope equipped with a temperature gradient-forming device, a simple tool for maintaining a closed, temperature-controlled, anoxic environment (Mora et al. 2014). Flagella are not only involved in cell motility, but also in adhesion to various surfaces and formation of cell-cell connections, resulting in a network of interconnected cells (Näther et al, 2006).
Objectives: As the mode of action of *P. furiosus* flagellar bundles is barely understood, we aim to investigate structure-function relationships in cells of these microorganisms. We are in the course of investigating the cellular architecture, the structural organisation of the flagella anchor, and the architecture of the S-Layer, in the wildtype and in laboratory strains exhibiting altered extent of flagellation (Näther-Schindler et al., 2014).
Methods: Cells of wildtype and mutant strains are investigated using various electron microscopical methods, in combination with proteome analysis. In particular, 400 nm sections of high-pressure frozen, resin-embedded *P. furiosus* cells are analysed using serial section dual-axis (S)TEM tomography at 200 kV. Datasets are reconstructed automatically using IMOD (Mastronarde and Held, 2016). The 3D architecture of intact cells is investigated using electron cryo-tomography (300 kV) and subtomogram averaging. Cytoplasmic and membrane proteins are separated based in differential and gradient centrifugation techniques and analysed using standard protocols.
Results: Tomography datasets turned to be essential for revealing new structural features in intact *P. furiosus* cells. We have determined the structure of the macromolecular motor, a ring-shaped assembly surrounding a central barrel, at the base of each flagellum. Each motor is anchored to a sheet-like cytoplasmic structure reminiscent of chemoreceptor arrays and each flagellar filament protrudes from the surface through gaps in the s-layer.
Conclusion: Using a combination of electron (cryo)-tomography, single-particle electron microscopy, known Xray structures, biochemical and bioinformatics data, we are in the process to identify and localize the structural components of the entire flagellum. These data will pave the way to a new understanding of archaeal motility at extreme temperatures.

References
[1] Näther et al, 2006: J Bacteriol 188: 6915-6923
[2] Näther-Schindler et al, 2014: Front Microbiol 5: 695
[3] Mora et al 2014: Appl Environm Microbiol 80: 4764-4770
[4] Mastronarde and Held 2016: J Struct Biol: in press

Presentation: Wednesday, 8 March 2017 from 8:45 - 9:00 in room 5.

219/ARV
The human archaeome
C. Moissl-Eichinger*[1]
[1]*Medical University Graz, Internal Medicine, Graz, Austria*

Background and question: The human body is the home of numerous bacteria, but also the third domain of life, the Archaea, is an important component of the human microbiome. However, the detection of Archaea is often hindered due methodological problems, so that their presence, abundance and importance are largely underestimated. The goal of our work is a) to improve the methods for archaeal detection and b) to reveal the archaeal contribution to the human microbiome in terms of: abundance, diversity and function.
Methods: We use a combination of amplicon sequencing, quantitative PCR and infrared spectroscopy to obtain insights into the human archaeome.
Results: Our investigations of the human archaeome revealed a broad diversity of Archaea in the human intestine, lung, and nose - and on skin1. For instance, we provide evidence that Archaea are a constant but highly variable component of the human skin microbiome. We were able to show, that Archaea are more abundant on skin of human subjects older than 60 years or younger than 12 years. By comparing these data with spectroscopy data, we were able to link lower sebum levels and lipid content and thus reduced skin moisture with an increase in archaeal signatures. Additional amplicon sequencing of selected samples revealed the archaeome of human skin, being comprised of specific eury- and mainly thaumarchaeal taxa.
Conclusions: We assume, that Archaea have important roles in the body-microbiome interplay, and that their activity certainly affects human health and well-being. However, the clinical relevance of Archaea remains unclear and awaits further studies with respect to the pathogenic or salutogenic potential of Archaea associated with the human body.

References
[1] Probst, AJ; Auerbach, AK; Moissl-Eichinger, C. Archaea on human skin. PLoS One. 2013; 8(6):e65388-e65388

Presentation: Wednesday, 8 March 2017 from 9:00 - 9:15 in room 5.

220/ARV
RNA of *Methanosphaera stadtmanae* activates proinflammatory immune responses
C. Bang*[1], T. Vierbuchen[2], H. Heine[2], R. A. Schmitz[1]
[1]*Christian-Albrechts-University Kiel, Institute for General Microbiology, Kiel, Germany*
[2]*Research Center Borstel, Research Group Innate Immunity, Borstel, Germany*

Question: Although the methanoarchaeal strains *Methanosphaera stadtmanae* and *Methanobrevibacter smithii* are known to be part of the natural human microbiota, their impact on the human immune homeostasis has only rarely been examined. During earlier studies, we observed that different archaeal strains led to different immune responses in human monocyte-derived dendritic cells (moDCs). Based on these findings, our study mainly aims to elucidate the cellular receptors and mediators involved in signaling processes that lead to the observed immune activation.

Methods: The response of human epithelial as well as immune cells upon the exposure to *M. stadtmanae* and *M. smithii* was investigated by quantification of released cytokines, CLSM analysis, qRT-PCR and transcription analysis (microarray). Isolation and purification of archaeal cell components was performed in order to examine the respective involved archaeal-associated molecular pattern.
Results: In particular *M. stadtmanae* is able to induce a strong release of proinflammatory cytokines and type I interferons in innate immune cells. Our recent findings led to the suggestion that exposure of human immune cells to *M. stadtmanae* activates the NLRP3 inflammasome and thereby initiates a profound secretion of IL-1b. Furthermore, our experiments show that purified archaeal RNA but not DNA is highly immunogenic as it is able to induce secretion of TNF-a, IL-6, IL-12 and IL-1b in human peripheral blood mononuclear cells (PBMCs) and moDCs.
Conclusions: Overall, our findings strongly argue that methanogenic archaea and primarily their RNA are specifically recognized by the human immune system through innate pattern recognition receptors (PRRs). The discovered high immunogenic potential of *M. stadtmanae* might argue for its potential involvement in the development of systemic intestinal diseases.

Presentation: Wednesday, 8 March 2017 from 9:15 - 9:30 in room 5.

221/ARV
Biochemical mechanism of nitrate-dependent methane oxidation
S. Berger*[1,2], M. S. M. Jetten[1,2], C. U. Welte[1,2]
[1]*Radboud University, Microbiology, Nijmegen, Netherlands*
[2]*Soehngen Institute of Anaerobic Microbiology, Nijmegen, Netherlands*

Introduction: Methane is a potent greenhouse gas contributing about 20% to global warming. Large quantities of methane are released from anoxic habitats where it is produced by methanogenic archaea. However, methane can be used as a substrate by aerobic and anaerobic methanotrophic microorganisms. Thus, only 20-50 % of the produced methane eventually reaches the atmosphere. Since methanotrophs play an important role in mitigating methane emissions it is crucial to understand the underlying biochemical mechanisms.
Objectives: Aerobic methanotrophy is comparably well understood, however methanotrophy under anoxic conditions is not yet fully explored. Recently, methanotrophic archaea (*Candidatus* Methanoperedens nitroreducens) that oxidize methane with concomitant reduction of nitrate were discovered [1, 2]. These archaea do not depend on an obligate syntrophic partner. It was the main goal of this study to elucidate the mechanism of nitrate-dependent methane oxidation as catalyzed by Methanoperedens-like archaea.
Materials and methods: Methanoperedens-like archaea are grown in a 10 L settled batch reactor with minimal medium and continuous supply of methane and nitrate. For enzymatic assays cells are harvested anoxically, lysed and separated in membrane and cytoplasmic fraction. The membrane fraction is used in spectrophotometric assays with $F_{420}H_2$ and different electron acceptors.
Results: Metagenome and transcriptome data revealed that in Methanoperedens-like archaea methane is oxidized via reverse methanogenesis [3, 4]. Reducing equivalents generated in reverse methanogenesis are presumably oxidized via an $F_{420}H_2$ dehydrogenase, a Rieske-cytochrome *b* complex and a Nar-type nitrate reductase [3, 4]. This study provides first experimental evidence for presence and activity of the membrane-bound enzymes of the respiratory chain.
Conclusion: This study provides insight into the biochemical mechanism of nitrate-dependent methane oxidation. To our knowledge, this is the first project targeting the functional potential of anaerobic methanotrophic archaea going beyond environmental and genomics techniques.

References
[1] Raghoebarsing et al. (2006) Nature 440, doi:10.1038/nature04617
[2] Haroon et al. (2013) Nature 500, doi:10.1038/nature12375
[3] Arshad et al. (2015) Front Microbiol 6, doi:10.3389/fmicb.2015.01423
[4] Welte et al. (2016) Environ Microbiol Rep 8, doi:10.1111/1758-2229.12487

Presentation: Wednesday, 8 March 2017 from 9:30 - 9:45 in room 5.

222/ARV
Same same but different: Structure-function analysis of an archaeal Argonaute variant with a novel activity
S. Willkomm*[1], A. Zander[1], C. Oellig[2], S. Schneider[2], D. Grohmann[1]
[1]*Universität Regensburg, Lehrstuhl für Mikrobiologie, Regensburg, Germany*
[2]*Technische Universität München, Center for Integrated Protein Science Munich CIPSM, München, Germany*

Argonaute (Ago) proteins from all three domains of life are key players in processes that specifically regulate cellular nucleic acid levels (1,2). Some of these Ago proteins, among them human Argonaute2 (hAgo2) and Ago from the archaeal organism *Methanocaldcoccus jannaschii* (MjAgo), are able to cleave nucleic acid target strands that are recognised via an Ago-associated complementary guide strand. While MjAgo binds DNA and RNA strands, it exclusively cleaves DNA substrates (3,4). We determined the full-length crystal structure of MjAgo in the absence of a DNA substrate, which exhibits high similarity to the eukaryotic Ago structure but also shows unknown structural features that influence Argonaute function. Furthermore, we determined the MjAgo structure bound to a DNA guide which reveals hitherto unknown conformational changes of Ago upon loading of the DNA guide. MjAgo features a putative second nucleic acid binding channel, which is essential for efficient cleavage.
We furthermore discovered that MjAgo operates in two modes of action exhibiting the canonical DNA-guided DNA endonuclease activity and the novel non-guided endonuclease activity. The latter allows MjAgo to process long double stranded DNAs, including circular plasmid DNAs and genomic DNAs. This is the first-time characterisation of a guide-independent silencing activity for an Argonaute protein potentially serving as guide biogenesis pathway in a prokaryotic system.

References
[1] Swarts D.C., Makarova K., Wang Y., Nakanishi K., Ketting R.F., Koonin E.V., Patel D.J., van der Oost J. (2014) The evolutionary journey of Argonaute proteins. Nat Struct Mol Biol 21:743-53.
[2] Willkomm S., Zander A., Gust A., Grohmann D. (2015). A prokaryotic twist on Argonaute action. Life. 5 (1), 538-553.
[3] Zander A., Holzmeister P., Klose D., Tinnefeld P. and Grohmann D. (2014). Single-molecule FRET supports the two-state model of Argonaute action. RNA Biology 11(1):45-56.
[4] Willkomm S., Zander A., Grohmann D. and Restle T. (2016). Mechanistic insights into archaeal and human Argonaute substrate binding and cleavage properties. PLOS one, 11(10):e0164695

Presentation: Wednesday, 8 March 2017 from 9:45 - 10:00 in room 5.

WORKSHOP 35
Allgemeine Hygiene, Krankenhaushygiene und Infektionsprävention im öffentlichen Gesundheitsdienst (FG PR / StAG HY)
08 March 2017 • 08:30 – 10:00

223/HYV

Importation of giardiasis into Germany by travelers, migrants and deployed soldiers – an issue of concern?

R. M. Hagen*[1,2], D. Wiemer[1], W. Maaßen[1], H. Frickmann[1,3]
[1]*Bundeswehr Hospital Hamburg, Department of Tropical Medicine at the Bernhard Nocht Institute, Hamburg, Germany*
[2]*Bundeswehr Medical Academy, Department of Preventive Medicine, Munich, Germany*
[3]*University Medicine Rostock, Institute for Medical Microbiology, Virology and Hygiene, Rostock, Germany*

Introduction: Giardiasis is an infectious disease caused by *Giardia duodenalis*, a protozoan parasite that is transmitted via the fecal-oral route in case of problematic hygienic conditions. In 2015, the incidence was 4.5 per 100,000 inhabitants in Germany. Although autochthonous transmission occurs in Germany, cases are frequently imported from abroad as travel-associated infectious diseases. Migration, international travel and military deployments are potential sources of influx of *Giardia duodenalis* into Germany, but the quantitative dimension of this phenomenon is poorly assessed so far.
Methods: PCR-based screening for *Giardia duodenalis* is established at the Department of Tropical Medicine at the Bernhard Nocht Institute, Bundeswehr Hospital Hamburg. Stool samples from a total of 528 civilian travelers, 158 migrants and 830 German soldiers after deployment as well as from a control group of 292 non-deployed German soldiers were assessed for this pathogen. As a control group from a high endemicity setting, samples from 410 asymptomatic volunteers from the highlands of Madagascar were included.
Results: A total of 37 out of 528 civilian travelers (7%) were positive for *Giardia duodenalis*. From those 37 travelers, 21 had returned from South Asia with a focus on the Indian subcontinent (18 returnees from India). Among the migrants, 20 out of 158 (12.7%) were positive for *Giardia duodenalis*, with highest infestation rates in migrants from Eritrea (21.4%) and Afghanistan (14.1%). Among returning German soldiers, 16 out of 830 (1.9%) were positive, while 2 out of 292 (0.7%) detections were observed among the non-deployed soldiers. Particular high risk was confirmed for UN-observers in Sudan (4.7%), reflecting their need to live outside controlled field camps and to consume food and water from local sources. From 410 asymptomatic Madagascan volunteers, 210 (51.2%) were positive for *Giardia duodenalis*.
Discussion: Among international travelers, migrants and returning soldiers, the infestation rate was considerably higher than in the German population. Especially for returning soldiers, a clear-cut association with living outside controlled field-camp infrastructure was identified as a risk factor. Because infestations are frequently asymptomatic after continued exposition in high-endemicity settings, importation of *Giardia duodenalis* into Germany can go undetected if no screening is performed, posing a risk of fecal-oral transmission with further spread of the pathogen.

Presentation: Wednesday, 8 March 2017 from 8:30 - 8:48 in room 12.

224/HYV

Pyoderma outbreak among families of kindergarten children: Association with a Panton-Valentine leucocidin (PVL)-producing *S. aureus* clone

R. Leistner*[1], R. Krüger[2], S. Schneider-Burrus[3], F. Layer[4], L. G. Hanitsch[5]
[1]*Charite Universitätsmedizin Berlin, Hygiene and Environmental Medizine, Berlin, Germany*
[2]*Charite Universitätsmedizin Berlin, Department of Pediatric Pulmonology and Immunology, Berlin, Germany*
[3]*Charite Universitätsmedizin Berlin, Department of Dermatology and Allergy, Berlin, Germany*
[4]*Robert Koch Institut, National Reference Center for Staphylococci and Enterococci, Wernigerode, Germany*
[5]*Charite Universitätsmedizin Berlin, Institute of Medical Immunology, Berlin, Germany*

Staphylococcus aureus strains carrying a gene for Panton-Valentine leucocidin (PVL) have been linked to massive skin abscesses in otherwise healthy individuals. We report of an outbreak of pyoderma due to PVL-positive MSSA and its control among families of children of an urban kindergarten in Berlin, Germany.
We established a multidisciplinary outbreak team, started a questionnaire-based epidemiological analysis and a microbiological screening examination of all individuals associated with the kindergarten. The found PVL-positive S. aureus isolates were further analyzed by whole genome sequencing. In order to discontinue the outbreak the affected families were started on *S. aureus* decolonization.
The results showed a clonal outbreak of PVL-positive methisillin-susceptible *S. aureus* (MSSA) with resistance to cothrimoxazole. We found 6 individuals colonized but without any history of pyoderma. There was a strong genetical relation to a clone of a former outbreak in another country outside of Germany. One colonized but not infected kindergarten family reported regular travel to the same area. The majority of the affected families were successfully decolonized. After the initiation of the decolonization no new case of pyoderma or colonization was found.
Our investigation showed that PVL-positive *S. aureus* strains can be introduced by international travel. Colonization with PVL-positive MSSA often does not lead to symptomatic infections. Pyoderma outbreaks due to this pathogen can successfully be controlled by *S. aureus* decolonization of all individuals at risk.

Presentation: Wednesday, 8 March 2017 from 8:48 - 9:06 in room 12.

225/PRV

Experimental colonisation of piglets with livestock-associated MRSA via the airborne route

K. Rosen *[1], F. Ebner [2], A. Friese [1], U. Roesler [1]
[1]*Institute for Animal Hygiene and Environmental Health, Freie Universität Berlin , Berlin, Germany*
[2]*Institute of Immunology, Freie Universität Berlin , Berlin, Germany*

Introduction: *Staphylococcus (S.) aureus* can colonise human and animal skin and mucosa without causing any clinical signs. Nevertheless, Methicillin-resistant *S. aureus* is able to cause severe infections. Since 2004, MRSA was often found in livestock -predominantly the specific sequence type ST398. The so called livestock-associated (LA-) MRSA was detected particularly in pig farms (Voss et al., 2005). So far, the transmission pathways between animals, from animals to humans and vice versa are not investigated in detail. Since MRSA was proven in air samples, an airborne route is discussed.
Objectives: The aim of our study is to evaluate the required dose for a successful colonisation of piglets with MRSA ST398 via the airborne way. Furthermore, we want to define risk factors which could influence the target dose.
Materials & methods: To answer these questions, groups of nine MRSA-negative piglets each were exposed in an aerosol chamber

with defined MRSA concentration in conditioned air. We aerosolised a low, middle and high concentration (102 to 106 cfu/m3) of a LA-MRSA strain isolated from a healthy pig and characterised as MLST ST398. During the aerosol exposure time of 24 hours, air samples were collected three times via impingement to verify the desired LA-MRSA concentration in air. After that, different animal swab samples were taken (nasal, skin, pharyngeal, conjunctival and rectal swab) three times a week for a period of 21 days to monitor the LA-MRSA colonisation. To examine the presence of MRSA in the internal organs, necropsy was done at the end of the screening period. A group was defined as successful colonised when at least 70% of the animals where LA-MRSA-positive for a time period of 14 days.
Results: The first group was exposed with the lowest MRSA concentration (102 cfu/m3). In this group only one animal was positive directly after staying in the chamber. In the second group (104 cfu/m3) all animals were positive after the exposition. Then the number of positive swabs varied until day 16. After that, the swab samples remained negative. The last group was exposed with the high load of MRSA (106 cfu/m3). All animals of that group were MRSA-positive for at least one kind of swab sample for the whole screening period.
Conclusion: Our study was able to confirm the assumption that an airborne route of MRSA exists. Whereas group two showed a transient colonisation the animals of group three were successful permanent colonised. The mean MRSA concentration in barn air is about 102 cfu/m3. So there is a large difference between this finding and our target MRSA dose. This discrepancy could be explained with the duration of our MRSA exposition which is just for 24 hours. Other factors like endotoxins or corrosive gases (CO2, NH3, H2S) in the air of pig barns could influence the necessary dose for a permanent colonisation. The effect of a suppressed immune system are currently examined and these data will be also presented.

Presentation: Wednesday, 8 March 2017 from 9:06 - 9:24 in room 12.

226/PRV
Do Antimicrobial Resistant Bacteria Spread in the Commercial Kitchen Environment after introduction via contaminated food? - The example MRSA.
A. Fetsch*[1], B. Ballhausen[1], P. Hiller[1], A. Kaesbohrer[1]
[1]*German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany*

Question: An expansive spread of antimicrobial resistant bacteria with livestock origin such as Methicillin-resistant *Staphylococcus aureus* (MRSA) has been shown in the past. At present, the extent to which food of animal origin contributes to the occurrence of human infections with MRSA cannot be quantified. MRSA frequently enter the kitchen environment via food (e.g. chicken/turkey meat). The level of MRSA cross-contamination in the kitchen currently remains unknown hindering reliable risk assessments. Therefore, the aim of this study was to investigate the spread of MRSA in a commercial kitchen environment after introduction via contaminated poultry meat.
Methods: Three different commercial kitchens located in the Berlin/Brandenburg area and owned by the same caterer were included in the study. Each kitchen was visited twice and when raw turkey/broiler meat was further processed for food preparation, only. Sampling was done as follows: (I) before, (II) during, and (III) after preparation of raw meat. Swab samples were taken from different positions of the kitchen environment (e.g. handles, fittings, control panels, cooking equipment etc.). In addition, food samples from raw poultry meat and thawing water were taken. Samples were further processed using selective enrichment methods. Microbiological analysis targeted MRSA and *S. aureus* as indicator organism. Suspected isolates were confirmed by MALDI-TOF and multiplex real-time PCR; *S. aureus* were further *spa*-typed and characterized by a DNA-microarray.
Results: In total, 259 samples were taken. MRSA were detected only once (in raw poultry meat). *S. aureus* as indicator organism were detected at 5/6 sampling visits, 14 different sampling positions (including 2/6 raw poultry meat samples) and at all three (I-III) sampling time points. Molecular analysis revealed highly identical *S. aureus* isolates in raw meat/thawing water from poultry and in samples taken from kitchen equipment used during handling of poultry meat, such as tongues, gloves worn by personnel, or tubs during 3/6 sampling visits. *S. aureus* belonging to other genetic lineages adapted to humans (clonal complex CC5 and CC8) were also found in the kitchen environment.
Conclusions: Antimicrobial resistant bacteria such as MRSA do enter commercial kitchens via contaminated raw poultry meat. Their spread seems to be limited to positions which are directly linked to the processing/preparation of raw poultry meat as shown by findings of *S. aureus*. Results underline the necessity of strict separation of working areas and equipment used in commercial kitchens. Whether kitchen personnel are a source of antimicrobial resistant bacteria found in the environment need to be further investigated.

Presentation: Wednesday, 8 March 2017 from 9:24 - 9:42 in room 12.

227/PRV
Occurrence of multidrug-resistant bacteria in municipal and hospital wastewater and receiving surface waters
C. Schreiber*[1], H. Müller[1], T. Kistemann[1], M. Exner[2]
[1]*Institute for Hygiene and Public Health (IHPH), University of Bonn, GeoHealth Centre, Bonn, Germany*
[2]*Institute for Hygiene and Public Health (IHPH), University of Bonn, Bonn, Germany*

Antibiotic-resistant intestinal bacteria can enter the environment through sanitary facilities and sewage treatment plants. Some multiply or survive in the environment, and persisting genes can be transferred to other microorganisms. Man and animal may become colonized with these bacteria if they are exposed to surface water. Infections caused by antibiotic-resistant bacteria are difficult to treat effectively with antibiotics. The worldwide increase in antibiotic-resistant bacteria is considered as a major challenge by the WHO and was a topic at the G7 summit in 2015.
To minimize the spread of antibiotics or antibiotic-resistant bacteria into the environment, the dissemination routes must be characterized and evaluated. The transfer of antibiotic resistance genes as well as the contamination of the environment with antibiotics or antibiotic degradation products has also to be considered.
The multi-disciplinary joint project "Biological and hygienic–medical relevance and control of antibiotic-resistant pathogens in clinical, agricultural and municipal waste water and their relevance in raw water (HyReKA)" funded by the Federal Ministry of Education and Research (BMBF), Germany (FKZ 02WRS1377A), studies these aspects while trying to answer the question: Which impact has the spread of these bacteria by different pathways for our health?
Possible ways of entry of multidrug-resistant bacteria into the environment, especially into surface waters, under investigation (Source Dissemination) are sewage from hospitals, abattoirs, agro-industrial holdings, airports, municipal wastewater, combined and separated sewer systems, reduction in sewage treatment plants and discharge by the effluent and landscape waters (run-off). Traceability between source and environment in terms of Microbial Source Tracking will be tested.
Feedback effects from the environment back to humans by contact to wastewater or surface waters (e.g. access to sanitary facilities by hospital patients and staff or access to recreational waters by

public) in terms of Microbial Dissemination will be included in risk assessment.
Beside identification of the most important sources or pathways, results shall support specifically outbreak control of antibiotic resistant pathogens in hospitals. Here, gram negative ESBL-producing bacteria, VRE and MRSA are of special interest. In contrast to former projects, a combination of classical culturing analysis, molecular methods and chemical analysis of antibiotic contents is used to identify risk potential.
This presentation will give an insight into the case studies investigated by the GeoHealth Centre (IHPH) concerning the pathways hospital and municipal wastewater based on the current status of the project HyReKA and discusses possible consequences for practice.

Presentation: Wednesday, 8 March 2017 from 9:42 - 10:00 in room 12.

WORKSHOP 36
Update on Fungal Research (FG EK / SL FB)
08 March 2017 • 08:30 – 10:00

228/EKV
Expression, processing, and secretion of the Ece1 polyprotein and its derived peptide toxin Candidalysin

S. Mogavero*[1], S. Allert[1], L. Kasper[1], T. Krüger[2], J. Richardson[3], J. Naglik[3], B. Hube[1,4,5]
[1]*Hans Knöll Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*
[2]*Hans Knöll Institute, Molecular and Applied Microbiology, Jena, Germany*
[3]*Dental Institute, King's College London, Mucosal and Salivary Biology Division, London, United Kingdom*
[4]*Friedrich Schiller University, Jena, Germany*
[5]*Center for Sepsis Control and Care, Integrated Research and Treatment Center, Jena, Germany*

Question: *Candida albicans*, an important opportunistic fungal pathogen of men, is able to transition from the yeast morphology, usually associated with the commensal life style in the gut, to a filamentous hyphal state. This filamentous morphology has long been associated with the invasive and destructive stage of *C. albicans*, and is accompanied by the expression of several hyphae-associated genes. Among these, *ECE1* is one of the most highly transcribed during hyphal growth. The role of *ECE1* during mucosal infection has recently been elucidated: it encodes a polyprotein that is processed by Golgi-associated Kex proteases into several peptides, which are then secreted in the extracellular space. One of these is a peptide toxin, Candidalysin, which is able to damage human host cells.
Of all Ece1 peptides, Candidalysin seems to be the first to be secreted and the most abundant in supernatants. Open questions remain pertaining the dynamics of expression, processing and secretion of the Ece1 peptides, as important steps preceding pore-forming mediated damage of epithelial cells by Candidalysin, and are the focus of this study.
Methods: To understand the dynamics of expression, processing and secretion, we performed a systematic screening of several mutants defective in damage potential and/or hyphal formation and/or that are linked to Ece1 processing. The mutants were screened for the following features: quantification of *ECE1* transcripts by RT-qPCR, ability to cause damage of oral epithelial cells using cytotoxicity assays, ability to form hyphae by measuring hyphal length, and composition of the secretome by LC-MS analysis.
Results: We identified mutants with reduced or no *ECE1* expression, with lower or no ability to form hyphae, secreting less or no Ece1-derived peptides and with reduced or no damaging potential. Low damage, compared to the wild-type, correlated with low *ECE1* expression and/or shorter hyphae and/or mutated *ECE1* sequences, especially around the Candidalysin sequence. Of note, mutants, where specific KR cleavage sites recognized by Kex2 had been modified to KA, lost their ability to properly release Candidalysin and to damage host cells.
Conclusions: *C. albicans* uses its hyphal morphology to cause damage to host cells, and this is mediated by the Candidalysin peptide toxin. It is clear that, whenever at least one of the two requirements fails, or does not reach a specific threshold, the fungus is handicapped and unable to cause damage. This goes both ways, that is, whenever a mutant is unable to cause damage, the reason will likely be an indirect effect of reduced *ECE1* expression or reduced delivery of Candidalysin to the host cells, either because hyphae are shorter, or because the processing or secretion is somehow impaired. In agreement with this conclusion, an optimal ratio of hyphal invasion, hyphal length and Candidalysin secretion is required for full damage.

Presentation: Wednesday, 8 March 2017 from 8:30 - 8:45 in room 7-8.

229/EKV
Biofilm formation of *Exophiala dermatitidis* and its susceptibility to antiinfective agents

L. Kirchhoff*[1], M. Olsowski[1], L. Sedlacek[2], G. Haase[2,3], J. Buer[1], P. M. Rath[1], J. Steinmann[1]
[1]*Medical Microbiology, University Hospital Essen, Essen, Germany*
[2]*Hannover Medical School, Institute of Medical Microbiology , Hannover, Germany*
[3]*Rheinisch-Westfälische Technische Hochschule Aachen University Hospital, Institute of Medical Microbiology, Aachen, Germany*

Because biofilms are a source of recurrent and chronic bacterial and fungal infections, the clinical importance of their prevention and treatment is increasing. One fungus that frequently colonises the respiratory tract of cystic fibrosis (CF) patients is the opportunistic black yeast–like fungus *Exophiala dermatitidis*. We investigated the biofilm-forming ability of *E. dermatitidis* and its susceptibility to various antiinfective agents and natural compounds. We tested 58 *E. dermatitidis* isolates with a biofilm assay based on crystal violet staining. In addition, we used three isolates to examine the antibiofilm activity of voriconazole, micafungin, colistin, farnesol, and the plant derivatives 1,2,3,4,6-pentagallolyl glucose (PGG) and epigallocatechin-3-gallate (EGCG) with an XTT reduction assay. We analysed the effect of the agents on cell to surface adhesion, biofilm formation, and the mature biofilm. The biofilms were also investigated by confocal laser scan microscopy. We found that *E. dermatitidis* builds biofilm in a strain-specific manner and that invasive *E. dermatitidis* isolates form most biomass in biofilm. The antiinfective agents and the natural compounds exhibited poor antibiofilm activity. The greatest impact of the compounds was detected when they were used for prevention. These findings suggest that prevention may be more effective than treatment of biofilm-associated *E. dermatitidis* infections.

Presentation: Wednesday, 8 March 2017 from 8:45 - 9:00 in room 7-8.

230/EKV
Characterization of the type III histidine kinase of *Aspergillus fumigatus*, which represents an interesting target structure for the development of new anti-fungal agents.

F. Ebel[1], A. Spadinger*[1], A. Wiedemann[1]
[1]*LMU, Institut für Infektionsmedizin und Zoonosen, Munich, Germany*

The High Osmolarity Glycerol (HOG) pathway is a signaling cascade allowing fungi to thrive under hyperosmotic conditions. In many fungi, the sensing of hyperosmotic stress is mediated by a so-called type III histidine kinase and in *Aspergillus fumigatus* is

the sole representative of this family of signaling kinases is TcsC. Type III histidine kinases can also be activated artificially by certain agents, which results in a dramatic imbalance of the internal and external osmotic pressure. We have recently analyzed the impact of these agents on *A. fumigatus* in more detail and found that activation of TcsC also results not only in a dramatic swelling and lysis of hyphal cells, but also in an immediate halt of the hyphal growth, a dramatic reorganization of the fungal cell wall, a closure of all septal pores and an uncontrolled proliferation of the nuclei. The observed fungicidal effect makes type III histidine kinases an attractive target for new anti-fungal agents, in particular since these kinases are found in most pathogenic fungi, but not in mammals.
All type III histidine kinases consist of a C-terminal kinase module and an N-terminal sensing module, which harbors several so-called HAMP domains. To unravel the functional relationship between both modules, we have expressed a GFP fusion of the TcsC kinase module in *A. fumigatus* using a inducible tet-on promoter system. The resulting mutant, when activated by doxycyclin, showed a dramatic phenotype similar to that found after pharmacological activation of TcsC. Expression of a similar construct which additionally comprises the sixth HAMP domain lacked this constitutive activity. Hence, the sensing module of TcsC is negatively regulated by the HAMP6 domain, a finding that is in contrast to the results of several studies on type III histidine kinases in yeasts. Surprisingly, expression of the GFP-HAMP6-sensing module fusion in the wild type resulted in a phenotype that resembled that of a *tcs*C deletion mutant, demonstrating that this fusion protein confers a dominant negative effect on native TcsC. Further data will be presented demonstrating the functional importance of other elements of the complex architecture of the TcsC kinase.

Presentation: Wednesday, 8 March 2017 from 9:00 - 9:15 in room 7-8.

231/FBV

Green Light sensing in *Fusarium fujikuroi*: investigation of the fungal rhodopsins CarO and OpsA

J. Avalos[1], J. Pardo[1], A. Adam[2], S. Deimel[2], S. Sputh[2], U. Terpitz*[2]
[1]*University of Seville, Genetics, Sevilla, Spain*
[2]*Julius Maximilian University, Biotechnology and Biophysics, Wuerzburg, Germany*

Question: Various filamentous fungi sense green light via membrane-standing microbial rhodopsins. Up to now, not many details are known about the physiological function and biological role of fungal rhodopsins. *Fusarium fujikuroi* contains two rhodopsin encoding genes, *carO* and *opsA*. The *carO* gene is linked to and co-regulated with genes coding for enzymes for retinal synthesis, whose expression is strongly induced by light. Also the *opsA* gene is upregulated by light. While the research in bacterial rhodopsins has a long history, in contrast, the information about fungal rhodopsins is small and their biological function is almost unknown. In the present work we aim to gain more insights into the role of rhodopsins and their importance for fungi.
Methods: In order to figure out their possible biological role, we fused CarO and OpsA to fluorescent proteins and expressed the fusion constructs in *F. fujikuroi* mycelia, yeasts, and mammalian cells, which were analyzed by confocal laser scanning microscopy (cLSM) and patch-clamp techniques.
Results: We observed that in light exposed mycelia CarO and OpsA are mainly expressed in conidia but to some extend also in growing hyphae. CarO is an efficient proton pump. In contrast, OpsA does not provide any net charge transfer under those conditions which is in accordance with observations in the related fungal rhodopsin nop-1 from *Neurospora crassa*. We found that CarO retards the conidial germination in light. Analyzing the dynamics of conidia germination of rhodopsin-deficient strains by a customized image-J plugin, we obtained data suggesting a faster and earlier germination of the CarO deletion mutant. During phylogenetic analysis we noticed high abundance of fungal rhodopsin in phyto-associated fungi suggesting a potential role of these green-light sensors in the plant-fungus interaction. We investigated this aspect in rice-infection experiments with rhodopsin-deficient fungal strains giving more evidence to this hypothesis.
Conclusions: The fungal rhodopsins of *F. fujikuroi* are highly expressed in the conidia where CarO was shown to be a proton pump that slows down germination. Our recent data suggest an involvement of the green light-sensing fungal rhodopsins in the plant-fungus interaction.

References

[1] Estrada, A.F. & Avalos, J. 2009. *J. Mol. Biol.* 387(1):59–73. García-Martínez, J., Brunk, M., Avalos, J. & Terpitz, U. 2015. *Sci. Rep.* 5:7798.
[2] Prado, M.M., Prado-Cabrero, A., Fernández-Martín, R. & Avalos, J. 2004. *Curr. Genet.* 46(1):47–58.

Presentation: Wednesday, 8 March 2017 from 9:15 - 9:30 in room 7-8.

232/FBV

Phosphoproteomic identification of a functional phosphosignaling network in fungi

R. Märker*[1], A. Beier[1], B. Blank-Landeshammer[2], A. Sickmann[2], U. Kück[1]
[1]*Ruhr-Universität Bochum, LS Allgemeine und Molekulare Botanik, Bochum, Germany*
[2]*Leibniz-Institut für Analytische Wissenschaften-ISAS, Dortmund, Germany*

Question: Fungi have a high relevance as human, animal and plant pathogens as well as producers of pharmaceutical products. Further, they are excellent experimental systems to analyze fundamental biological processes in eukaryotes due to their short life cycle and easy genetic manipulation. *Sordaria macrospora* is a well-established model fungus to study the formation of multicellular structures, such as fruiting bodies. This developmental process is controlled by subunits of the highly conserved striatin-interacting phosphatase and kinase (STRIPAK) complex present in fungi and animals. In fungi, this complex is involved in sexual development, hyphal fusion and vegetative growth. Further, it putatively forms a regulatory network with various other signaling pathways. In *S. macrospora*, the STRIPAK complex comprises several core components, including protein phosphatase 2A (PP2A), which consists of the structural subunit PP2AA, the regulatory subunit PRO11 and the catalytic subunit PP2Ac1.
We are interested in identifying the target proteins that are phosphorylated or dephosphorylated by the STRIPAK complex to get a detailed insight into the regulatory mechanism of this complex during diverse eukaryotic developmental processes.
Methods: We performed mass spectrometry-based proteome and phosphoproteome analysis of the wild type and mutant strains pro11, Δpp2Ac1 and Δpro22, in order to unravel phosphorylation-based signal transduction by the STRIPAK complex. Besides, we characterized the proteome and phosphoproteome of the double deletion strains Δpp2Ac1Δpro22 and Δpro11Δpro22 in comparison to Δpro11 and the wild type.
Results: We identified putative phospho-targets of the STRIPAK complex, which are related to different signaling pathways and involved in the sexual development of *S. macrospora*. Among these target proteins, we identified a regulator of the NADPH oxidases and a scaffold protein of the pheromone response MAP kinase pathway.

Conclusion: Our data suggest that the STRIPAK complex is critical for the function of signal transduction pathways by protein phosphorylation/dephosphorylation. These results will increase our knowledge about the mechanistic function of STRIPAK in eukaryotic multicellular processes.

Presentation: Wednesday, 8 March 2017 from 9:30 - 9:45 in room 7-8.

233/FBV

Enzyme production with *Aspergillus nidulans* under growth limited conditions in a trickle bed reactor

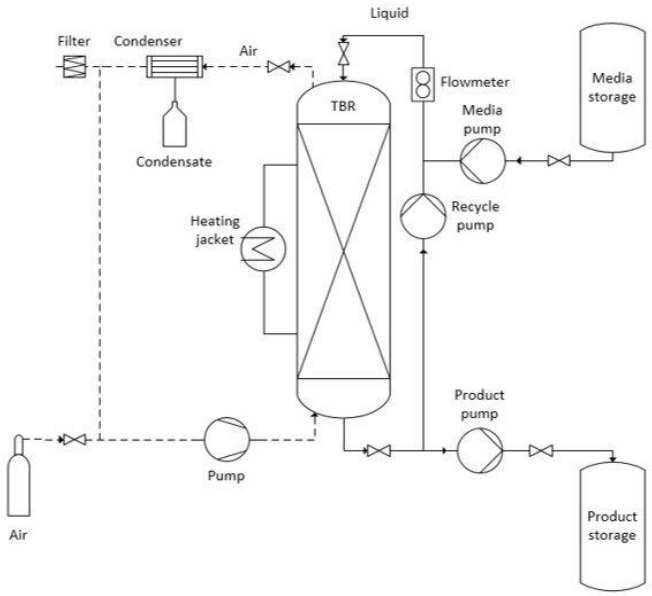
M. Müller*[1], O. Pardo Planas[2], P. Ballmann[1], M. Wilkins[3], R. Prade[4], S. Dröge[1]
[1]*Prüf- und Forschungsinstitut Pirmasens e.V., Biotechnology and Microbiology, Pirmasens, Germany*
[2]*Oklahoma State University, Biosystems and Agricultural Engineering, Stillwater, Oklahoma, United States*
[3]*University of Nebraska-Lincoln, Biological Systems Engineering, Lincoln, Nebraska, United States*
[4]*Oklahoma State University, Microbiology and Molecular Genetics, Stillwater, Oklahoma, United States*

Enzymes play a key role in the transition towards a biobased economy, allowing reactions to be catalyzed at milder conditions and with a higher level of specificity. The greatest challenge of increased utilization of enzymes is their cost. According to a study in 2012, the costs vary between $0.68 and $1.47 per gallon of ethanol [1]. Submerged fermentation (SmF) is the most used way of enzyme production. However, filamentous fungi may lead to problems in SmF, such as fouling of equipment and high viscosity with subsequent mass transfer issues. An alternative is solid state fermentation (SSF), which reportedly leads to improved volumetric productivities, but suffers from poor mixing and lack of continuous operation. Trickle bed reactors (TBR) combine the best from both worlds. A solid support provides a surface for the fungus to grow while media with nutrients trickles through the reactor column. A major drawback is the uncontrolled growth of mycelia in reactor components in contact with the fermentation broth, which can cause clogging. To address this problem, it was proposed to limit growth during enzyme production. Aspergillus nidulans strains are available with selection markers. A strain with a pyridoxine marker requires pyridoxine in the medium for growth. As was shown in previous studies, a strain grown in a TBR produced the target enzyme continuously, even after the required coenzyme pyridoxine was removed from the media [2, 3]. In the present study, the growth-limited production of aryl-alcohol oxidase (AAO) was optimized using a reduced pyridoxine concentration. The fermentation was demonstrated in a 4-liter lab-scale TBR (Fig. 1). Different packing materials were tested. Dilution rate, aeration, and recycle rate were optimized and found to be 0.034 h-1, 0.1 vvm and 150 ml/min, respectively. The system allowed a continuous AAO production over 350 h with production rates of up to 3.0 U/ml*h. No differences in enzyme production were found for the different packing materials. The strain exhibited excessive melanin formation, which could be solved by reduced air supply and addition of 5 g/l ascorbic acid. Activities for scale-up to 300 liters are ongoing. The results showed a successful continuous enzyme production under limit growth conditions with the potential to simplify downstream processing and reduce costs.

References

[1] Klein-Marcuschamer, D., et al., The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnology and bioengineering, 2012. 109(4): p. 1083-1087.
[2] Müller, M., et al., High-yield recombinant xylanase production by Aspergillus nidulans under pyridoxine limitation. J Ind Microbiol Biotechnol (2014) 41: 1563.
[3] Müller, M., et al., Continuous xylanase production with Aspergillus nidulans under pyridoxine limitation using a trickle bed reactor. Biorecource Technology (2015) 188:219-25

Figure 1: Schematic of TBR for continuous enzyme production.
Figure 1



Presentation: Wednesday, 8 March 2017 from 9:45 - 10:00 in room 7-8.

WORKSHOP 37
Infection Immunology (FG II)
08 March 2017 • 08:30 – 10:00

234/IIV

***Mycobacterium tuberculosis* survival upon efferocytosis of infected neutrophils by macrophages requires ESAT-6**

T. Dallenga*[1,2], U. Repnik[3], R. Reimer[4,2], G. Griffiths[3], U. Schaible[1,2]
[1]*Research Center Borstel, Cellular Microbiology, Borstel, Germany*
[2]*German Centre for Infection Research, Borstel, Germany*
[3]*University of Oslo, Oslo, Norway*
[4]*Heinrich-Pette-Institute, Core Facility Microscopy & Image Analysis, Hamburg, Germany*

Extensively and multidrug-resistant isolates of *Mycobacterium tuberculosis* are on the rise worldwide. Novel host-directed therapies are promising adjunct measures to antibiotics treatment. Polymorphonuclear neutrophils (PMN) represent the main infected cell population in lungs of patients with active tuberculosis, but fail to kill *M. tuberculosis*. Instead, virulent but not RD1-deficient mycobacteria induce necrotic cell death in a reactive oxygen species (ROS)-dependent manner and escape from neutrophil killing. Macrophages remove dead neutrophils in a process termed efferocytosis. We studied the role of efferocytic removal of mycobacteria-infected neutrophils in macrophage defense against *M. tuberculosis*. We found that efferocytosis of necrotic neutrophils infected with wild type *M. tuberculosis* promoted mycobacterial survival and growth. Necrosis of infected neutrophils was a prerequisite for mycobacterial survival upon efferocytosis. In contrast, mutants lacking either the RD1 region or the RD1-encoded protein ESAT-6 alone did not induce neutrophil necrosis and subsequently failed to grow in efferocytes. Pharmacological prevention of *M. tuberculosis*-induced, ROS-mediated PMN necrosis led to growth control of mycobacteria by efferocytic macrophages. Taken together, the decision whether efferocytosed *M. tuberculosis* survived in macrophages was

determined by the presence of ESAT-6, which set the course for differential PMN cell death pathways and intracellular trafficking routes in and cell death of efferocytes, thereby, ultimately, promoting infection. Our data emphasize the detrimental role of neutrophils and the type of cell death for tuberculosis pathology and disease outcome, making them a predestined candidate for both, point of care testing as well as target for host-directed therapies.

Figure 1

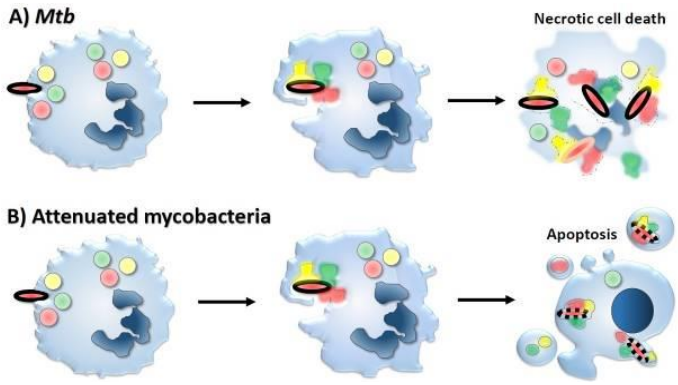
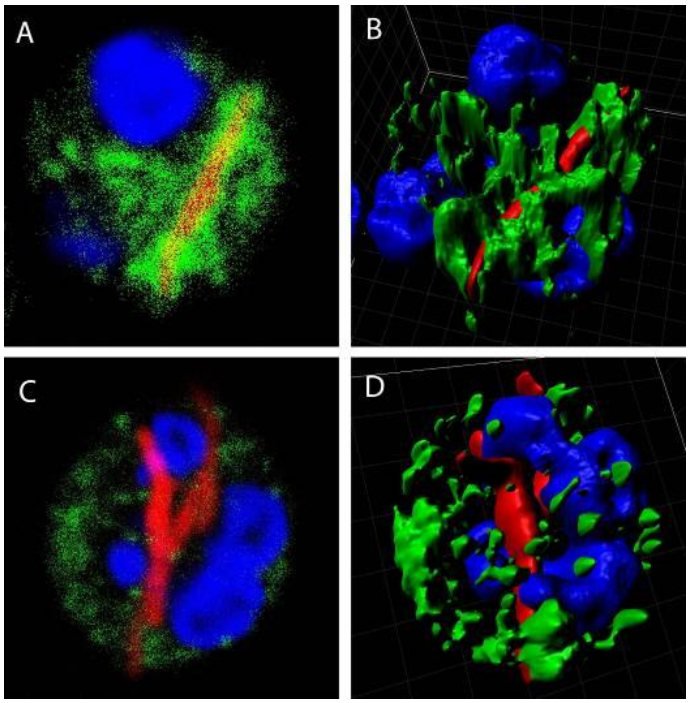


Figure 2



Presentation: Wednesday, 8 March 2017 from 8:30 - 8:45 in room 6.

235/IIV

Dermal Fibroblasts Play a Central Role in Skin Model Protection against *C. albicans* Invasion

A. Kühbacher*[1], P. Stevens[1,2], C. Grumaz[1], H. Henkel[1], D. Finkelmeier[1], A. Burger-Kentischer[1,3], K. Sohn[1,3], R. Steffen[1,3]
[1]*Fraunhofer IGB, Molecular Biotechnology, Stuttgart, Germany*
[2]*Medical University of Vienna, Vienna, Austria*
[3]*University of Stuttgart, Stuttgart, Germany*

Research efforts in the last decades have shed light on mechanistic interactions of fungal pathogens with host organisms down to the molecular level. Although considerable progress has been made in the last decades, the function of the individual components of epithelial barriers with regard to host-microbial interaction is not fully understood.
The fungal pathogen *Candida albicans* colonizes basically all human epithelial surfaces including the skin. Under certain conditions, such as immunosuppression, invasion of the normally protected epithelia occurs. In natural skin these infections are only superficial but not much is known about defense mechanisms against *C. albicans* in subepithelial layers such as the dermis.
Using immune cell-supplemented 3D skin models we could define a new role for fibroblasts in the dermis and identify a minimal set of cell types for skin protection against *C. albicans* invasion. Dual RNA-Seq of individual host cell populations and *C. albicans* revealed that dermal invasion is directly impeded by dermal fibroblasts. They are able to integrate signals from the pathogen and CD4+ T cells and shift towards an antimicrobial phenotype with broad specificity that is dependent on TLR2 and IL-1β. Skin model protection could be induced by addition of IL-1β even in the absence of T cells. TLR2 activation in dermal fibroblasts which occurs through *C. albicans*, results in induction of IL-1β expression, but it is secreted only in the presence of CD4+ T cells. These results highlight a central function of dermal fibroblasts for skin protection opening new possibilities for treatment of infectious diseases.

Presentation: Wednesday, 8 March 2017 from 8:45 - 9:00 in room 6.

236/IIV

Global antibody response to *Staphylococcus aureus* live-cell vaccination

T. Hertlein*[1], M. Selle[1], P. Kloppot[2], E. Müller[3,4], R. Ehricht[3,4], B. Bröker[5], S. Engelmann[6], K. Ohlsen[1]
[1]*University Wuerzburg, Institute for Molecular Infection Biology, Wuerzburg, Germany*
[2]*University Greifswald, Institute for Microbiology, Greifswald, Germany*
[3]*Alere Technologies GmbH, Jena, Germany*
[4]*InfectoGnostics Research Campus, Jena, Germany*
[5]*University Medicine Greifswald, Department of Immunology, Greifswald, Germany*
[6]*Helmholtz Centre for Infection Research, Braunschweig, Germany*

The pathogen *Staphylococcus aureus* is well-known for the broad range of severe diseases it can cause and feared for its ability to rapidly develop resistance to antibiotics. Particularly the increasing number of highly resistant and/or community-acquired *S. aureus* infections urges the search for alternative treatment options such as vaccination or passive immunization to close the widening gap in anti-*S. aureus* therapy.
This study focussed on the characterization of the humoral immune response to vaccination of Balb/c mice with sublethal doses of live *S. aureus*. The elicited antibody pattern in the sera of intravenously (i.v.) and intramuscularly (i.m.) vaccinated mice was determined using of a recently developed protein array. Interestingly, we observed a specific antibody response against a broad set of *S. aureus* antigens which was stronger following i.v. than i.m. vaccination. It differed furthermore in the specificities of the generated antibodies, indicating variations in either staphylococcal gene expression or the accessibility of antigens for

the immune. Moreover, only intravenous but not intramuscular vaccination led to superior outcome in an intramuscular infection with a high bacterial dose. This might suggest that antibody levels and/or specificities influence the effective immune response against *S. aureus in vivo*.
In a second set of experiments we tested whether deletion of selected virulence-associated genes of *S. aureus* influences the humoral immune response of the host. We found to our surprise, that these factors were not only able to modulate the level of antibodies generated by the host (in this case, the mouse), but also the isotypes, thus interfering with the efficacy of the antibody response against bacterial challenge in terms of quantity and quality.
To sum up, this study identified on one hand novel vaccine candidates by using protein microarrays as an effective tool and showed that successful vaccination against *S. aureus* relies on the optimal route of administration. On the other hand, it could demonstrate that *S. aureus* interferes massively with antibody generation in the host, what has to be taken into account for future immunization approaches.

Presentation: Wednesday, 8 March 2017 from 9:00 - 9:15 in room 6.

237/IIV
Dual role of the mycobacterial cord factor TDM in cross-regulation of macrophage responses to IFNγ
B. Killy*[1], A. Huber[1], A. B. Ekici[2], S. Wirtz[3], R. Lang[1]
[1]*Universitätsklinikum Erlangen, Klinische Mikrobiologie, Erlangen, Germany*
[2]*Universitätsklinikum Erlangen, Humangenetik, Erlangen, Germany*
[3]*Universitätsklinikum Erlangen, Medizinische Fakultät 1, Erlangen, Germany*

Mycobacterium tuberculosis (MTB) manages to survive and replicate in macrophages at least in part through inhibition of IFNγ-induced responses. The most abundant mycobacterial cell wall glycolipid is trehalose-6,6-dimycolate (TDM), also known as the cord factor. TDM is recognized by the C-type lectin receptor Mincle leading to macrophage activation. Here we investigated how the cord factor shapes the response to IFNγ. Global transcriptome analysis revealed both synergistic and antagonistic effects of TDM on IFNγ-stimulated gene expression in murine macrophages. For instance, co-stimulation with TDM further boosted IFNγ-induced NOS2 expression, whereas MHCII antigen presentation and elicitation of T cell responses were reduced. In addition, TDM-Mincle dampened induction of Gbp1, which is critical for resistance to several intracellular bacteria. Regarding the mechanisms underlying TDM mediated gene-specific impairment of IFNγ responses, we found that IFNγR expression, proximal IFNγR signaling as well as induction of IRF1, a pivotal transcription factor in IFNγ signaling, were not affected. TDM acts on the transcriptional level because Gbp1 primary transcript expression was inhibited. IFNγ-induced Gbp1 expression was attenuated in *Irf1-/-* macrophages, but not further reduced by TDM, indicating that gene-specific inhibition by the cord factor involves IRF1 activity. Together, our data reveals an ambiguous role of the cord factor in the cross-regulation of IFNγ signaling, as it synergistically promotes IFNγ-induced inflammatory chemokine and iNOS expression in macrophages, but, on the other hand, antagonizes MHCII antigen presentation and anti-microbial GTPase expression. By this transcriptional reprogramming of IFNγ responses, cord factor may contribute to mycobacterial immune evasion.

Presentation: Wednesday, 8 March 2017 from 9:15 - 9:30 in room 6.

238/IIV
Staphylococcus aureus protease Jep – a novel virulence factor?
H. El Gohary*[1,2], P. Trübe[1], J. Dick[1], J. Gumz[1], E. Rüdiger[1], T. Yang[3], S. Johnson[3], K. Schmoeckel[1], B. Bröker[1], S. Wiles[3], S. Holtfreter[1]
[1]*Universitätsmedizin Greifswald, Immunology, Greifswald, Germany*
[2]*Cairo University, Clinical Pathology, Cairo, Egypt*
[3]*University of Auckland, Bioluminescent Superbugs Lab, Auckland, New Zealand*

Question: The high incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) strengthens the need for new effective antibiotics and a protective vaccine. Up till now, mainly human-adapted S*taphylococcus aureus* strains were used to study *S. aureus* pathogenicity in mouse models. However, it is known that *S. aureus* is highly host-specific. Recently, a mouse-adapted S. aureus strain, JSNZ, was identified. This strain could be a promising tool in developing more appropriate infection models.
One third of JSNZ total protein production is a putative serine protease. It was named JSNZ extracellular protease (Jep). Bacterial extracellular proteases are known to degrade host tissue, facilitate bacterial spread and manipulate host immune responses. Similarly Jep could be important for colonization and infection. The aim of this study was to elucidate the role of Jep in the interaction between S. aureus and its host.
Methods: The sequence and location of the *jep* gene was determined with whole genome sequencing. The prevalence of *jep* in human and murine *S. aureus* isolates was then analyzed by PCR. A *jep* knockout mutant and a complemented strain were generated by chromosomal replacement. JSNZ and the derived genetically modified strains currently undergo functional assays, including intracellular survival and whole blood survival assays.
Results: The *jep* gene demonstrated up to 48% sequence homology to *S. aureus* serine protease-like proteins (SplA-F). Genome analysis of JSNZ showed that *jep* is located on a Sa1int phage within the virulence module, implying Jep is a virulence factor. The *jep* gene was present in over a quarter of the murine strains that were tested but was found to be very rare in human S. aureus isolates. The *jep* knockout strain showed a strongly reduced survival in murine whole blood compared to its wild type counterpart. This leads to the hypothesis that Jep manipulates the innate immune system. We are currently testing whether Jep interferes with neutrophil function, complement activation or antimicrobial peptides.
Conclusion: The data suggest that Jep manipulates the murine immune response. The underlying mechanisms remain to be clarified. Further studies using peptide libraries and substrate screening will provide insights into the specificity of the Jep enzyme and its role in *S. aureus* colonization and infection.

Presentation: Wednesday, 8 March 2017 from 9:30 - 9:45 in room 6.

239/IIV
Cross talk between T cells and antigen presenting cells during M*ycobacterium avium* complex infection
K. A. Merga*[1], A. Nerlich[1], N. Ruangkiattikul[1], S. Weiss[2], R. Goethe[1]
[1]*Institute for Microbiology, University of Veterinary Medicine Hannover, Center for Infection Medicine, Hannover, Germany*
[2]*Helmholtz Center for Infection Research, Molecular Immunology, Braunschweig, Germany*

Background: *Mycobacterium avium* complex represents a group of genetically closely related bacteria with different, specific phenotypic and genotypic features. *M. avium* subspecies *paratuberculosis* (MAP) is the causative agent of paratuberculosis (Johnes disease), a chronic intestinal disease, in cattle and other ruminants. MAP is also suggested as the possible cause of Crohns disease, a persistent inflammation of the bowel in humans. MAP is distinguished from its closest relatives *M. avium* ssp. *avium*

(MAA) and *M. avium* ssp. *hominissuis* (MAH) as it exhibits a strong intestinal tropism. There is strong evidence that MAP escapes local immune surveillance to establish long term infection leading to clinical disease. However, only little is known about the underlying immunological mechanism.
Objectives: The presented study was performed to analyze the functional capacity and phenotypes of antigen presenting cells (APCs) and T cell response after MAP infection of mice in comparison to infections with the closely related MAA.
Methods: We established *ex vivo* and *in vivo* antigen specific T cell proliferation assays. C57BL/6 mice were infected with MAP or MAA. At different time points after intraperitoneal infection, splenic dendritic cells (DCs) were sorted and co-cultured with CD4 T cells from OT II TCR transgenic mice in the presence ovalbumin protein or peptide. Similarly, antigen specific proliferation was measured *in vivo* after adoptive transfer of OT-II CD4 T cells.
Results: Our *ex vivo* and *in vivo* experiments showed that MAA but not MAP infection of mice affected the antigen presenting capacity of splenic DCs resulting in the inhibition of antigen specific CD4 T cell proliferation. Antigen presenting cells co-stimulatory and co-inhibitory molecules were differently regulated. MAA infection perturbs myeloid cells compartment for survival while inducing B cells and T cells loss. However this is not observed after MAP infection.
Conclusion: Overall, these data show that MAP and MAA are distinct in their immune escape mechanism in mice. They reveal that MAP escapes the host immune response while MAA seems to actively subvert the host immune response.

Presentation: Wednesday, 8 March 2017 from 9:45 - 10:00 in room 6.

PLENARY 07
Antibiotics and Resistance
08 March 2017 • 10:30 – 12:00

240/INV
Evolution of Resistance to Ciprofloxacin in *Escherichia coli*.
D. Hughes*[1], D. L. Huseby[1], F. Pietsch[1], G. Brandis[1], L. Garoff[1], A. Tegehall[1]
[1]*Uppsala University, Department of Medical Biochemistry & Microbiology,Biomedical Center, Uppsala, Sweden*

Ciprofloxacin is a clinically important antibacterial drug targeting Type II topoisomerases and it is highly active against Gram-negatives including *Escherichia coli*. The evolution of resistance to ciprofloxacin in *E. coli* always requires multiple genetic changes, usually including mutations affecting two different drug target genes, *gyrA* and *parC*. Resistant mutants selected *in vitro* or *in vivo* can have many different mutations in target genes and efflux regulator genes that contribute to resistance. Among resistant clinical isolates the genotype, *gyrA* S83L D87N, *parC* S80I is significantly overrepresented suggesting that it has a selective advantage. However, the evolutionary or functional significance of this high frequency resistance genotype is not fully understood. By combining experimental data and mathematical modelling, we addressed the reasons for the predominance of this specific genotype. We measured drug susceptibility, mutation rates, and competitive fitness using isogenic strains carrying clinically relevant resistance mutations. The experimental data were used to model trajectories of mutational resistance evolution under different conditions of drug exposure and population bottlenecks. We identified the order in which specific mutations are selected in the clinical genotype, showed that the high frequency genotype could be selected over a range of drug selective pressures, and that its probability of selection was strongly influenced by the relative fitness of alternative mutations and factors affecting mutation supply. Our data map for the first time the fitness landscape that constrains the evolutionary trajectories taken during the development of clinical resistance to ciprofloxacin and explain the predominance of the most frequently selected genotype.

Presentation: Wednesday, 8 March 2017 from 10:30 - 11:00 in the Franconia Hall.

241/INV
Innovative Antibiotics from Microbes: Identification, Mode of Action and Resistance Mechanisms
R. Müller*[1]
[1]*Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Saarbruecken, Germany*

Microorganisms are accepted as a valuable source for chemical biology tools and drug leads. An outstanding group of microorganisms is represented by the ubiquitous myxobacteria, a largely underexploited resource for natural products (NP). Analyzing the few myxobacterial genomes known to date leads to the conclusion that they contain up to 50 respective biosynthetic gene loci per strain indicating the enormous genomic potential for the production of NPs. However, only few compounds are typically known per microbial isolate. In a comprehensive MS-based study we have addressed the question of compound diversity in correlation with phylogenetic diversity and found clear and measurable evidence that new compounds are most likely to be found in currently uncharacterized microbial genera and families.
Once novel microbial NPs are identified, their potential as antibiotic drug leads is analyzed by defining pharmaceutical properties and potential resistance mechanisms of pathogens against the compound. Target identification, which is usually a complex and rather unpredictable biochemical research endeavour, has become essential for drug development for numerous reasons including the possibility to rationally optimize lead compounds based on their molecular structure-target-complex. During the last decades genomics has become an integral part of NP drug research and allows not only for directed approaches to discover new natural products: Target identification might be achieved by studying self-resistance mechanisms within the producer strains or, alternatively, by defining the molecular basis of resistance in pathogens by whole genome sequencing of evolved bacterial resistance.
The presentation will cover some examples of novel antibiotics and target identification of microbial NPs.

References
[1] T. Hoffmann, D. Krug et al. Bacterial versus Chemical Diversity: The Phylogeny Paradigm in Microbial Natural Product Discovery. submitted
[2] S. Baumann, J. Herrmann, et al. Angew. Chem. IE 2014, 53, 14605–14609.
[3] A. Kling, P. Lukat, et al., Science 2015, 348, 1106–1112.
[4] F. Surup, K. Viehrig, et al., Angew. Chem. IE 2014, 49, 13588–13591.
[5] J. Herrmann, A. A. Fayad, et al., Nat. Prod. Rep. 2016, advance article, DOI:10.1039/c6np00106h.
[6] R. Müller, J. Wink, Int. J. Med. Microbiol. 2014, 304, 3-13.

Presentation: Wednesday, 8 March 2017 from 11:00 - 11:30 in the Franconia Hall.

242/INV
Antibiotic tolerance and persistence facilitate the evolution of resistance
N. Balaban*[1]
[1]*Hebrew University Jerusalem, Jerusalem, Israel*

Controlled experimental evolution during antibiotic treatment can shed light on the processes leading to antibiotic resistance in bacteria. Recently, intermittent antibiotic exposures have been shown to lead rapidly to the evolution of tolerance, i.e. the ability to survive under treatment without developing resistance. A special case of tolerance is persistence, in which only a small sub-population is tolerant. Both can contribute to survival under antibiotic treatment. However, whether tolerance and persistence delay or promote the eventual emergence of resistance is unclear. Here, we used *in vitro* evolution experiments to explore this question. We found that in all cases tolerance or persistence preceded resistance. A mathematical population-genetics model showed how tolerance boosts the chances for resistance mutations to spread in the population. Thus, tolerance mutations pave the way for the rapid subsequent evolution of resistance. Preventing the evolution of tolerance may offer a new strategy for delaying the emergence of resistance.

Presentation: Wednesday, 8 March 2017 from 11:30 - 12:00 in the Franconia Hall.

SHORT LECTURE 40
Metabolism and Biochemistry (SL EE)
08 March 2017 • 13:30 – 15:30

243/EEV
A flavin-binding protein is essential for the anaerobic decarboxylation of *o*-phthalyl-CoA by *Azoarcus* sp. strain PA01
M. Junghare*[1], D. Spiteller[1], B. Schink[1]
[1]*University of Konstanz, Biology , Konstanz, Germany*

o-Phthalic acid (1,2-dicarboxybenzene) is a man-made compound, whose esters are used globally in large quantities for a wide range of applications, mainly as plasticizers. Although, the anaerobic degradation of phthalate was known for decades, metabolites, enzymes and genes involved in the degradation were still unclear, particularly for the step of anoxic phthalate decarboxylation to benzoate. Here, we illustrate how the nitrate-reducing, *Azoarcus* sp. strain PA01 decarboxylates phthalate to benzoate during its anaerobic degradation. Differential two-dimensional protein profiling allowed the identification of specifically induced proteins in *o*-phthalate-grown versus benzoate-grown cells. Phthalate-induced genes were found to be placed in a single gene cluster in the genome of strain PA01 (1), coding for five proteins, a transporter, two CoA-transferase, and UbiD-like and UbiX-like decarboxylase, respectively. We suggest that *o*-phthalate is first activated to *o*-phthalyl-CoA by the succinyl-CoA-dependent succinyl-CoA:*o*-phthalate CoA-transferase and is subsequently decarboxylated to benzoyl-CoA by the *o*-phthalyl-CoA decarboxylase. *In vitro* enzyme assays with cell-free extracts of strain PA01 cells grown with *o*-phthalate demonstrated the formation of *o*-phthalyl-CoA, specifically with *o*-phthalate and succinyl-CoA as CoA donor, and established its subsequent decarboxylation to benzoyl-CoA using LC-MS analysis (2). Interestingly, phylogenetic analysis of two proteins PhtDa (PA01_00217) and PhtDb (PA01_00218) involved in *o*-phthalyl-CoA decarboxylation revealed, that they belong to a recently discovered enzyme family of UbiD-like and UbiX-like decarboxylases that function in ubiquinone synthesis in a wide range of bacteria, e.g., *E. coli* (3, 4). Cloning and heterologous expression of two proteins PhtDa (60 kDa) and PhtDb (23 kDa) in *E. coli* revealed, that each protein has a specific role in catalysing the *o*-phthalate decarboxylation anoxically. PhtDb is a flavin mononucleotide (FMN) binding protein that does not possess decarboxylase activity alone, but rather catalyses the formation of a modified FMN cofactor which is required by PhtDa for decarboxylation of *o*-phthalyl-CoA to benzoyl-CoA. Protein sequence alignment and computational structural modelling of both proteins suggested that, only PthDb has the binding site for FMN similar to the UbiX, a flavin-binding protein in *E. coli* (5).

References
[1] Junghare, et al., (2015). *Stand Genomic Sci* 10: 90.
[2] Junghare, et al., (2016). *Environ Microbiol* 18: 3175-3188.
[3] Gulmezian, et al., (2007). *Arch. Biochem. Biophys*. 467: 144-153.
[4] Kopec, et al., (2011). *Acta Crystallogr* 67: 1184-1188.
[5] White, et al., (2016). *Nature* 522: 502.

Presentation: Wednesday, 8 March 2017 from 13:30 - 13:45 in the Franconia Hall.

244/EEV
Novel insights in rhamnolipid biosynthesis
A. Wittgens*[1], F. Kovacic[2], M. Henkel[3], R. Hausmann[3], F. Rosenau[1]
[1]*Ulm University, Center for Peptide Pharmaceuticals, Ulm, Germany*
[2]*Heinrich-Heine-University Düsseldorf, Institute for Molecular Enzyme Technology (IMET), Jülich, Germany*
[3]*University of Hohenheim, Institute of Food Science and Biotechnology, Department of Bioprocess Engineering (150k), Stuttgart, Germany*

The biosurfactant rhamnolipid has various physiological roles and industrial applications. They feature a low toxicity and an enhanced biodegradability in comparison to detergents with petrochemical origin. The biosynthesis of rhamnolipids is well characterized for the opportunistic human pathogen *Pseudomonas aeruginosa.* They are composed of one or two rhamnose molecules linked through a β-glycosidic bond to a hydrophobic 3-hydroxyfatty acid moiety with various chain length. Due to the number of rhamnose sugars they were separated into mono- and di-rhamnolipids. The biosynthesis of rhamnolipids occurs in three enzymatic reactions: RhlA is responsible for the esterification of two 3-hydroxyfatty acids and synthesizes 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs). RhlB links a dTDP-L-rhamnose to the HAA and creates mono-rhamnolipids. So far RhlA and RhlB were described as two subunits forming a functional rhamnosyltransferase I enzyme complex, because respective genes are arranged as a bicistronic operon. In the last reaction the rhamnosyltransferase II RhlC links a second rhamnose molecule to the mono-rhamnolipids and synthesizes di-rhamnolipids. Bacteria predominantly produce rhamnolipid species containing two 3-hydroxyfatty acids although rhamnolipids containing only a single fatty acid chain were identified, too. The biosynthesis of these single fatty acid rhamnolipids is still speculative. Possibly, they descend from direct condensation of a dTDP-L-rhamnose with a 3-hydroxyfatty acid chain by RhlB or they could be produced by hydrolysis of one fatty acid of typical mono- and di-rhamnolipids by a still unknown enzyme.
To investigate the role of proteins involved in rhamnolipid biosynthesis the non-pathogenic *Pseudomonas putida* KT2440 was used as suitable host to circumvent the complex *quorum sensing* regulation in *P. aeruginosa*. *P. putida* provides both pathways essential for the production of 3-hydroxyfatty acids and dTDP-L-rhamnose used as rhamnolipid precursors.
Our results reveal that RhlA and RhlB fulfill their enzymatic functions independent from each other and not in form of a RhlAB heterodimeric complex as postulated previously. We could show that the synthesis of rhamnolipids containing only one 3-hydroxyfatty acid most likely occurs through hydrolysis of typical rhamnolipids containing two fatty acid chains rather than a side-

reaction in rhamnolipid biosynthesis. Furthermore, we demonstrate that exogenous mono-rhamnolipids and their precursors HAAs can be taken up by *P. putida* as well as *P. aeruginosa* and can be subsequently converted to di-rhamnolipids. These novel insights in the rhamnolipid biosynthesis pathway provide new approaches in synthesis of designer rhamnolipids.

Presentation: Wednesday, 8 March 2017 from 13:45 - 14:00 in the Franconia Hall.

245/EEV

Molecular mechanisms mediating tolerance to ionic liquids in *Listeria monocytogenes*

P. J. Mester[1], T. Gundolf*[1], R. Kalb[2], M. Wagner[1], P. Rossmanith[1]
[1]*Institute for Milk Hygiene, Vienna, Austria*
[2]*Proionic Gmbh, Grambach, Austria*

Introduction: Ionic liquids (ILs), a new solvent class solely composed of ions, have found their way into numerous industrial applications. Due to their highly variable structure and unique physiochemical properties, research utilizing this emerging new technology for biochemical applications is steadily increasing. Most (eco)toxicological studies of ILs therefore mainly focus on acute toxicity, biostability and biodegradation aiming for environmentally friendly ILs. The manifold variability of ILs, however also facilitates their use in fields of antimicrobial substances or disinfectants, where potential toxic properties are desired. Commonly used disinfectants are based on quaternary ammonium compounds (QACs), like benzalkonium chloride. Inadequate disinfection and presence of sublethal concentrations frequently lead to resistant bacterial species, which oppose a threat to the public health. Even though ILs have structural similarities to QACs, the physiochemical properties differ substantially and therefore ILs could help to overcome the deficiencies of classical disinfectants offering new alternatives.
Objectives: The objective of our work was to test different ILs towards bacteria regarding the bacterial response and compare the results to traditional QAC based substances in order to see if ILs, as novel class of antimicrobial substance, underlie the same mechanisms as conventional disinfectants. In our experimental approach the food- pathogen *L. monocytogenes*, known to have specific efflux pumps against QAC substances, was tested.
Material& Methods: Commercially available QACs (BC, DTAB, Domiphen bromide, Benzethonium chloride) and ILs based on imidazolium [Cnmim]Cl (n=2,4,6,8,10) and ammonium [TMCnA]X (n=4,8,12,16; X=Cl or maleate) cations were tested against *L. monocytogenes* strains (-Tn*6188*: CDL65, R479a, CDL2, CDL77, 535; +Tn*6188*: CDL78, 4423, N22-2, F17, 6179) in a serial dilution microtiter plate assay. Adaption to sub-lethal concentrations of QACs and ILs was investigated via a specific adaption process.
Results: Experiments indicated that *L. monocytogenes* strains harbouring transposon Tn*6188* (+Tn*6188*), not only had an elevated MIC against the tested QACs but also against the tested imidazolium and ammonium- based ILs. Exposing two *L. monocytogenes* strains (CDL2, 6179) to sub- lethal concentrations of BC and [C10mim]Cl could furthermore show that *L. monocytogenes* is not only able to adapt to higher concentrations of BC but also to higher concentrations of [C10mim]Cl.
Conclusion: These results are significant for both practical and fundamental reasons. For the first time it was demonstrated that ILs as a potential class of disinfectants succumb to the same resistance mechanisms in bacteria as conventional QACs. However, considering the immense IL variability it should be possible to develop ILs overcoming bacterial defence systems.

Presentation: Wednesday, 8 March 2017 from 14:00 - 14:15 in the Franconia Hall.

246/EEV

Complete dechlorination of tetrachloroethene to ethene by a co-culture of the organohalide-respiring organisms *Sulfurospirillum multivorans* and *Dehalococcoides mccartyi*

S. Kruse*[1], T. Goris[1], D. Türkowsky[2], N. Jehmlich[2], L. Adrian[3], M. Westermann[4], G. Diekert[1]
[1]*Friedrich Schiller University, Applied and Ecological Microbiology, Jena, Germany*
[2]*Helmholtz Centre for Environmental Research, Molecular Systems Biology, Leipzig, Germany*
[3]*Helmholtz Centre for Environmental Research, Isotope Biogeochemistry, Leipzig, Germany*
[4]*Friedrich Schiller University, Electron Microscopy Centre, Jena, Germany*

Introduction: One of the mostprominent groundwater pollutants, tetrachloroethene (PCE), can be dechlorinated anaerobically by specialized bacteria. This process is coupled to growth of the participating bacteria, in which the chlorinated ethenes are used as terminal electron acceptor, catalyzed by corrinoid-dependent reductive dehalogenases. However, many versatile, fast-growing PCE-dechlorinating bacteria, like the Epsilonproteobacterium *Sulfurospirillum multivorans*, are only able to dechlorinate PCE to the harmful dichloroethene (DCE) (1). Complete dechlorination to ethene is mainly achieved by *Dehalococcoides mccartyi*, a bacterium characterized by its obligate organohalide-respiratory lifestyle and its slow growth. Hydrogen is the only electron donor used by *D. mccartyi* in this process. In previous studies, co-cultures of *D. mccartyi* with other bacteria showed better growth and faster dechlorination rates than in pure cultures (2). However, a co-culture with a hydrogen-producing, rapidly PCE-dechlorinating bacterium was never tested. Since *S. multivorans* was recently shown to produce hydrogen under fermentative conditions, we set up two separate co-cultures of the *D. mccartyi* strains BTF08 and 195, each with *S. multivorans* transferring hydrogen, corrinoid, and acetate to *D. mccartyi*.
Methods: Dechlorination of chlorinated ethenes was measured gas-chromatographically. Field emission-scanning electron microscopy was used to visualize details of the co-culture. Comparative label-free quantitative proteomics was performed to get insights into adaptations of the organisms to their co-culture-specific lifestyle.
Results: Co-cultures amended with lactate as carbon and energy source and PCE as electron acceptor showed complete dechlorination of PCE to ethene, indicating hydrogen transfer from *S. multivorans* to *D. mccartyi*. On lactate alone, *S. multivorans* showed no growth. Since *D. mccartyi* relies on H2 as electron donor, this result implies an obligate syntrophic interaction between the two organisms. PCE to ethene dechlorination rates were more than twice as fast as in pure cultures. Besides hydrogen, also corrinoid was shown to be transferred, as reductive dechlorination occurred not in vitamin B12-free single cultures, but only in co-cultures.
Conclusion: In the rapidly PCE to ethene dechlorinating co-culture of *D. mccartyi* and *S. multivorans*, the latter acts as a swiss army knife: It not only produces hydrogen and acetate, used by *D. mccartyi* as energy and carbon source respectively, but also corrinoid as dehalogenase cofactor and DCE, which is further dechlorinated by *D. mccartyi* to ethene.

References

[1] Neumann *et al.*, *Arch. Microbiol.* (1994), doi: 10.1007/BF00301854
[2] Mao *et al.*, AEM (2015), doi: 10.1128/AEM.03464-14

Presentation: Wednesday, 8 March 2017 from 14:15 - 14:30 in the Franconia Hall.

247/EEV

Comparative genomic studies of *Desulfosporosinus*: functional diversity in anaerobic hydrocarbon-impacted environments

N. Abu Laban *[1,2], B. Tan [3], A. Dao[2], J. Foght [2]
[1]*University of Duisburg-Essen, Biofilm Centre, Essen, Germany*
[2]*University of Alberta, Department of Biological Sciences, Edmonton, Canada*
[3]*Singapore-MIT Alliance for Research and Technology, Singapore, Singapore*

Question: Members of the Gram-positive genus *Desulfosporosinus* are frequently detected in anoxic, hydrocarbon-impacted habitats. They are thought to be involved in the anaerobic degradation of aromatic hydrocarbons like toluene, ethylbenzene, and xylenes. Although four complete genome sequences of *Desulfosporosinus* strains (one of which degrades toluene) have been published, potential genes involved in hydrocarbon activation have not yet been described. Therefore, the potential metabolic roles of *Desulfosporosinus* in hydrocarbon-impacted environments remains unclear.
Methods: Comparative metagenomics was applied to two *Desulfosporosinus* genomes obtained from different methanogenic oil-impacted sites: one genome (*Dssp* SIP) was obtained from the metagenome of a toluene-degrading culture enriched from an oil sands tailings pond; the other genome (*Dssp* HMP52) was obtained from a pure strain isolated from a coal bed. The *Dssp* SIP and *Dssp* HMP52 Illumina Miseq raw DAN sequences were subjected to quality control and de novo assembly. Then, draft genomes were reconstructed by sequence binning using a composition-based method. The draft genomes of *Dssp* SIP (acc. JQID00000000) and *Dssp* HMP52 (acc. JMGA010000000) consisted of 228 and 84 contigs and were compared with the four *Desulfosporosinus* genomes published in NCBI (NC_018515.1, NC_016584.1, NC_018068.1, and NR_115694.1).
Results: Pan-genome sequence analysis of the accessory genes indicated that the *Dssp* SIP and *Dssp* HMP52 genomes were distinct from each other but that the latter sequence was closely related to the genome of *D. meridiei* strain DSM1325, indicating that the two new genomes represent different species, and *Dssp* SIP may be novel. More than 1,000 accessory open reading frames (ORFs) identified in the *Dssp* SIP genome are located in clusters comprising genes encoding for anaerobic activation of hydrocarbons, prophages, and transposases. About ~2,000 *Dssp* HMP52 accessory ORFs are likely to be involved in diverse functions including fermentative, anaerobic benzoate degradation and transport, flagellar biosynthesis and chemotaxis, replicative transposition, and dissimilatory sulfite reduction. Thus, we propose that *Dssp* SIP might function as a primary degrader of hydrocarbons in the absence of sulfate (i.e., under methanogenic conditions), whereas *Dssp* HMP52 might play a role in the secondary fermentation of hydrocarbon metabolites, syntrophy, and sulfate reduction.
Conclusions: Comparative genomics depicted a potential role for members of the genus *Desulfosporosinus* in the oil-impacted environments. The presence of mobile elements like transposases and prophage in the accessory genome suggests that horizontal gene transfer might have played a key role in this functional diversity.

Presentation: Wednesday, 8 March 2017 from 14:30 - 14:45 in the Franconia Hall.

248/EEV

Genomic repertoire and activity of the *Woeseiaceae*, cosmopolitan and abundant core members of microbial communities in marine sediments

M. Mussmann*[1], P. Pjevac[1]
[1]*University of Vienna, Division of Microbial Ecology, Vienna, Austria*

To date very little is known about the bacterial core community of marine sediments. Here, we study the environmental distribution, abundance and ecogenomics of the recently established gammaproteobacterial family *Woeseiaceae*. A meta-analysis of published 16S rRNA gene amplicon datasets shows that the *Woeseiaceae* are ubiquitous and consistently rank among the most abundant bacterial groups in diverse marine sediments. They account for up to 22% of bacterial amplicons and 6% of total cell counts in European and Australian coastal sediments. The analysis of a single cell genome, metagenomic bins and the genome of the next cultured relative *Woeseia oceani* indicated a broad physiological range including heterotrophy and facultative autotrophy. All tested (meta)genomes encode a truncated denitrification pathway to nitrous oxide. The broad range of energy-yielding metabolisms possibly explains the ubiquity and high abundance of *Woeseiaceae* in marine sediments, where they carry out diverse, but yet unknown ecological functions.

Presentation: Wednesday, 8 March 2017 from 14:45 - 15:00 in the Franconia Hall.

SHORT LECTURE 41
Single Cell Analysis and Infection Models (SL MCB)
08 March 2017 • 13:30 – 15:00

249/MCBV

***Chlamydia trachomatis* Prevents Mitochondrial Fragmentation Via The miR-30c-P53-Drp1 Axis**

R. S. Chowdhury*[1], A. Reimer[1], M. Sharan[2], V. Kozak-Pavlovic[1], A. Eulalio[2], B. Prusty[1], M. Fraunholz[1], K. Karunakaran[1], T. Rudel[1]
[1]*University of Wuerzburg, Derpartment of Microbiology, Wuerzburg, Germany*
[2]*University of Wuerzburg, Institute for Molecular Infection Biology (IMIB), Wuerzburg, Germany*

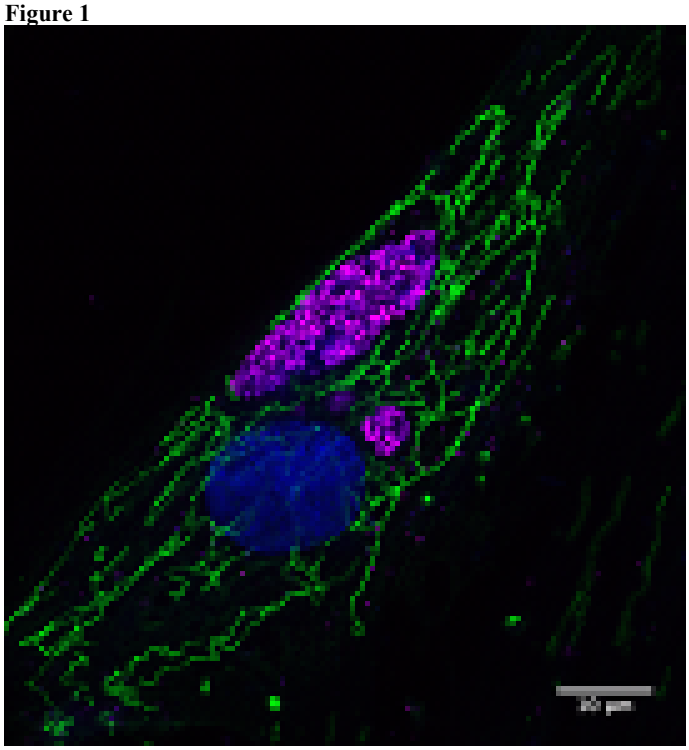
Question: Obligate intracellular bacteria like *Chlamydia* depend on metabolites of the host cell and thus, protect their sole replication niche by interfering with the host cells stress response[1]. Here, we investigated the involvement of host microRNAs (miRNAs) in maintaining the viability of *Chlamydia* infected primary human cells.
Methods: Using RNA sequencing and Northern blot analysis we identified a prominently upregulated miRNA required for the stable downregulation of p53, a major suppressor of metabolite supply in *Chlamydia*-infected primary cells[2]. Using live cell imaging in conjunction with a novel FIJI-based Macro script "MitoCRWLR" we quantified changes in the mitochondrial morphology of *Chlamydia* infected cells. We further used structured illumination microscopy to identify and compare the availability of mitochondrial fission sites between *Chlamydia* infected and non-infected cells. To determine the degree of stress experienced by the host mitochondria upon *Chlamydia* infection we quantified ROS mediated stress within the mitochondrial matrix using the stress sensitive florescent TIMER protein.
Results: We observed that the loss of the candidate microRNA led to an increase in the rate of mitochondrial fission, which in turn, severely affected chlamydial growth and had a marked effect on the integrity of the host mitochondrial network. Super resolution microscopy revealed that mitochondrial fragments undergo significant elongation (Figure 1) and exhibit a loss of

mitochondrial fission sites in *Chlamydia* infected cells. Furthermore, artificial induction of mitochondrial fragmentation prevented replication of *Chlamydia* even in several different primary cell lines. Our experiments with the TIMER protein showed that *Chlamydia* maintains mitochondrial integrity during ROS-induced stress that occurs naturally during infection.
Conclusion: We show that *Chlamydia* require mitochondrial ATP for normal development and hence postulate that they preserve mitochondrial integrity of the host cell via a microRNA-dependent inhibition of mitochondrial fission.

Figure 1: Structured Illumination micrograph of primary epithelial cells of human fallopian tube fimbriae after 30hrs of *Chlamydia* infection. *Chlamydia* stained with Alexa fluor 647 against cHSP60 (purple), mitochondrial presequence tagged GFP indicates the mitochondria (green) and DAPI for marking the nucleus (blue). Bar represents 10 µm.

References
[1] Moulder, J.W. 1991. Interaction of chlamydiae and host cells in vitro. *Microbiological reviews*. 55:143-190.
[2] Siegl, C., B.K. Prusty, K. Karunakaran, J. Wischhusen, and T. Rudel. 2014. Tumor suppressor p53 alters host cell metabolism to limit Chlamydia trachomatis infection. *Cell Rep*. 9:918-929.

Figure 1



Presentation: Wednesday, 8 March 2017 from 13:30 - 13:45 in the Barbarossa Hall.

250/MCBV
Single molecule localization and tracking of SPI2-T3SS effector proteins in *Salmonella enterica* infected cells
V. Göser*[1], B. Barlag[1], R. Kurre[2], C. Richter[3], M. Hensel[1]
[1]*University of Osnabrück, Microbiology, Osnabrück, Germany*
[2]*University of Osnabrück, Center for Advanced Light & Electron Microscopy, Osnabrück, Germany*
[3]*University of Osnabrück, Biophysics, Osnabrück, Germany*

Background: *S. enterica* manipulates various host cell processes. Among these, aggregation and tubulation of endosomal membrane vesicles leads to formation of *Salmonella*-induced filaments (SIFs). Responsible for this phenomenon are effector proteins translocated into the host cell via the *Salmonella* pathogenicity island 2 (SPI2)-encoded type III secretion system (T3SS). These effector proteins colocalize with SCV and SIFs. So far, the analyses of dynamics of translocation and intracellular trafficking of these effector proteins was hampered by lack of fluorescence tags compatible with T3SS translocation. We devised a new approach deploying genetically encoded self-labeling enzymes (HaloTag) and TIRF super-resolution microscopy (SRM) that enabled us to investigate the subcellular localization and dynamics of SPI2-T3SS effector proteins in living cells.
Methods: Effector proteins were fused to HaloTag, an enzyme tag which rapidly forms a covalent bond to its ligands. Labeling took place using 20 nM of a cell-permeable, reactive chloroalkane-based ligand for the HaloTag conjugated to tetramethylrhodamine (TMR). To first establish the functional secretion of effector proteins with HaloTag, HeLa cells were infected, labeled and fixed 8 h p.I. and the localization of effector proteins were determined using dSTORM SRM. Next, we deployed in live, infected cells tracking and localization microscopy (TALM) of selected proteins to reveal the precise localization, as well as the dynamics of a single effector protein in membranes of SIFs.
Results: We tested the translocation of various effector-HaloTag fusion proteins and for most proteins labelling suitable for SRM was observed. The HaloTag is compatible with translocation by the SPI2-T3SS, fusion proteins are functional as effectors and the HaloTag is functional as self-labeling enzyme after translocation into host cells. The localization of various SPI2 effector proteins could be determined in relation to SIFs in *Salmonella*-infected cells. Additionally, the mobility of selected effector proteins (SifA, PipB2, SseF) in SIFs was established employing live cell SRM. The effector proteins SifA and SseF show a similar mobility as the membrane protein LAMP1. In contrast PipB2 showed a distinct association to the SIF membrane.
Conclusion: Here we present a new tool to study the localization, translocation kinetics and dynamics of *Salmonella* effector proteins in infected cells. SPI2 effector proteins fused to a HaloTag can be functionally translocated in infected cells and localize to SCV and SIFs. Moreover it is possible to monitor a single effector protein in SIFs during infection. This new technique provides an opportunity to compare mobility and localization of different SPI2 effector proteins and consequently allows an interpretation of the interaction of effector proteins with host cell membranes and further target structures.

Presentation: Wednesday, 8 March 2017 from 13:45 - 14:00 in the Barbarossa Hall.

251/MCBV
Meningococcal ligands and molecular targets required for adhesion and penetration of the B-CSF barrier
S. Gomes*[1], A. Appelt-Menzel[1], H. Walles[1], M. Metzger[1], A. Schubert-Unkmeir[2]
[1]*University Hospital Würzburg, Tissue Engineering and Regenerative Medicine, Würzburg, Germany*
[2]*University of Würzburg , Institute of Hygiene and Microbiology, Würzburg, Germany*

Introduction: *N. meningitidis* (*Nm*) colonizes the nasopharynx mainly as a commensal, being carried asymptomatically by 5 to 10% of the healthy population in non-endemic times. In rare cases, it can cross the epithelium of the nasophanrynx, gain access to the bloodstream and cause severe septicemia and/or meningitis. One of the main factors that contribute for the pathogenicity of *Nm* is its ability to penetrate the vascular endothelial cell layer and infect the meninges. Although the role of adhesins and invasins in the virulence of *Nm* has been demonstrated, the mechanisms that govern meningoccocal penetration are still not fully understood. Various models have been established to study the *Nm*-host interaction using mostly *in vitro* cell culture models based on immortalized human cell lines. However, none of these

established cell lines provides an appropriate experimental resource to study *Nm* colonization and subsequent invasion and penetration of human endothelial surfaces.
Objectives: We aim to establish induced pluripotent stem (iPS) cell-derived endothelial cells (iPS-ECs) as a novel cellular *in vitro* model for *Nm* research and use these cells to develop a human *in vitro* 3D and an *in vitro* circulatory 2D model of the blood-cerebrospinal fluid (B-CSF) barrier, in order to study colonization and penetration of brain microvascular endothelial cells by *Nm*.
Materials & methods: Cell culture and infection assays
Human iPS cells were maintained in feeder-free conditions using Matrigel coating. Differentiation was induced using time-dependent alteration of biochemical stimuli in the culture medium for 8 days, after which the cells were dissociated, plated at a density of $1x10^6$ cells cm^{-2} in a coating mixture of 1:2:2 of fibronectin: collagen IV : ddH2O with supplemented endothelial cell medium. Gentamicin protection assays and immunofluorescence assays were performed 48 h later. Infections were carried out in the presence of 10% human serum with bacteria at a multiplicity of infection of 10 for gentamicin protection assays or 100 for immunofluorescence assays.
Results: Our results using iPS-ECs show that *Nm* (strain MC58 and the isogenic unencapsulated mutant MC58 *siaD*) is able to adhere and invade these cells, maintaining the same pattern of infection as with human brain microvascular endothelial cells, an immortalized cell line commonly used in *Nm* research. Notably, iPS-ECs reach TEER values up to 2000 Ω cm^{-2}, forming tight monolayers that better reflect the physiological properties of the brain endothelial barrier.
Conclusion: Our results indicate that iPS-ECs can potentially be used as a novel cellular model to study *Nm* invasion. Our next steps will be to perform full infection kinetics with several *Nm* strains, analyse cytokine secretion and tightness of the barrier during infection (TEER and/or FITC dextran measurements) and perform qPCR and transport studies.

Presentation: Wednesday, 8 March 2017 from 14:00 - 14:15 in the Barbarossa Hall.

252/MCBV
Novel tissue-engineered human 3D infection models to study pathogenesis of *Helicobacter pylori* and *Campylobacter jejuni*
M. Alzheimer*[1], S. L. Svensson[1], M. Schweinlin[2], M. Metzger[2], H. Walles[2], C. M. Sharma[1]
[1]*University of Würzburg, Research Center for Infectious Diseases, Würzburg, Germany*
[2]*University Hospital Würzburg, Tissue Engineering and Regenerative Medicine, Würzburg, Germany*

Helicobacter pylori and *Campylobacter jejuni* are two of the most prevalent bacterial pathogens of the human gastrointestinal tract. Current infection models for these pathogenic bacteria are often limited in their ability to reflect the *in-vivo* situation in the human host. While 2D cell culture models mostly lack tissue complexity, the infection outcome in animal models often differs from that which manifests in disease-susceptible humans. Thus, we have been employing tissue engineering techniques to establish novel gastrointestinal 3D tissue infection models that more closely recapitulate the micro-environment of the human intestine and stomach. These tissue models are based on acellularized extracellular matrix scaffolds (SISmuc = small intestinal submucosa; GSmuc = gastric submucosa), which are reseeded with human gastrointestinal cell lines. Dynamic cultivation conditions promote the development of a tight epithelial barrier. Furthermore, we have improved the models by building them from cells that can produce a mucus layer. Infection studies with the intestinal tissue model and different *C. jejuni* isolates showed that formation of an adherent mucus layer mediates increased protection from colonization and disruption of epithelial barrier function. Infection of the 3D tissue model with diverse *C. jejuni* mutant strains revealed adherence and internalization phenotypes that have not been apparent in conventional *in-vitro* 2D cell culture systems, but have been observed in animal studies. Using the novel gastric tissue model, we can track *H. pylori* colonization and transmigration in this new 3D environment by isolation of colony forming units as well as by immunohistochemical staining. In addition, we are currently developing a primary gastric tissue model based on cells isolated from human tissue samples. Overall, our novel 3D tissue infection models represent promising new tools to study how bacterial pathogens interact with an environment more reminiscent of native host tissue. Moreover, dual RNA-seq analysis in these models will reveal in-depth molecular crosstalk between host and pathogen.

Presentation: Wednesday, 8 March 2017 from 14:15 - 14:30 in the Barbarossa Hall.

253/MCBV
RNA-Sequencing of microbial subpopulations – Towards, systems-level analyses of phenotypic heterogeneity
R. Frhr. v. Boeselager*[1], T. Polen[1], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences IBG-1: Biotechnology, Jülich, Germany*

Introduction: Microbial populations are highly complex and optimal adaptation to the particular ecological niche depends on complex interactions and specialisation within microbial communities. But cellular variation extends further to the level of clonal populations sharing the same genotype while differentiating into phenotypic subpopulations. Thus, dramatic differences may exist at the single-cell level, which are obscured by standard bulk techniques. Therefore, it is necessary to develop novel tools for the analysis of microbial subpopulations which are highly relevant for addressing various questions in the field of microbial population dynamics [1].
Objectives: The aim of this study is the establishment of a novel workflow combining fluorescence-activated cell sorting (FACS) and RNA-Sequencing for the analysis of phenotypic subpopulations.
Methods: As a model for the establishment of the envisaged workflow, we studied activation of the cryptic prophage CGP3, which is a well-known phenomenon observed in subpopulations of *Corynebacterium glutamicum.* Frequency of CGP3 induction was modulated by interfering with the activity of a recently described silencer protein CgpS [2]. Fusion of a phage promoter to *eyfp* was used to visualize the phage induction by flow cytometry. Subpopulations were separated via fluorescence activated cell sorting. Different sorting conditions and cell numbers were tested. Finally the RNA of sorted cells was isolated and sequenced using an Illumina MiSeq.
Results: The phage reporter construct revealed a heterogeneous response with respect to the phage-induction. The chosen conditions resulted in the formation of two subpopulations. The overall fraction of the prophage-activated cells was successfully modulated by expression of the N-terminal oligomerisation domain of CgpS. Using FACS 10^3-10^6 cells were sorted for subsequent RNA-Seq analysis. The RNA preparation represented the most critical step of the respective workflow. Here, different cell lysis and RNA preparation protocols were compared. Overall, the transcriptome profile of sorted cells matched the unsorted bulk control. However, analysis of the prophage-activated subpopulations significantly improved resolution of the transcriptome data and revealed effects, which were obscured by the bulk approach. Further attempts focus on the analysis of cell sorting effects and the optimization of storage conditions.
Conclusion: Many techniques are available to visualize cell-to-cell variation within microbial populations, but obscure the reason of it. Here we present a novel workflow combining FACS and

RNA-Seq enabling to unravel differences in transcriptome profiles of microbial subpopulations.

References
[1] Jehmlich, N. *et al.*, (2010). Appl. Microbiol. Biotechnol., 88, 575–584.
[2] Pfeifer, E. *et al.*, (2016). Nucleic Acids Res., gkw692.

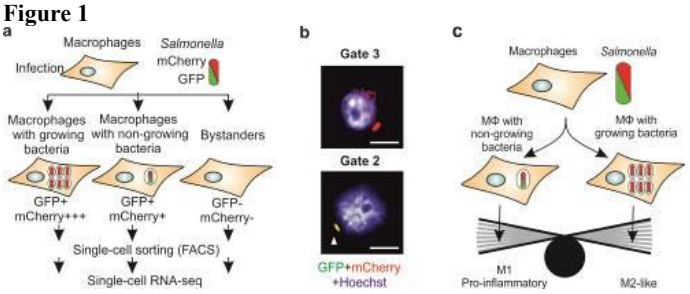
Presentation: Wednesday, 8 March 2017 from 14:30 - 14:45 in the Barbarossa Hall.

254MCBV
Single-cell RNA-seq ties macrophage polarization to growth rate of intracellular Salmonella
A. E. Saliba*[1], L. Li[1], A. Westermann[1], S. Appenzelle[1], D. Stapels[2], L. Schulte[1], S. Helaine[2], J. Vogel[1]
[1]*University of Wuerzburg, Institute of Molecular Infection Biology, Wuerzburg, Germany*
[2]*Imperial College London, Centre for Molecular Bacteriology and Infection, London, United Kingdom*

Intracellular bacterial pathogens can exhibit large heterogeneity in growth rate inside host cells with major consequences for the infection outcome. If and how the host responds to this heterogeneity remains poorly understood.
Here, we combined a fluorescent reporter of bacterial cell division with single-cell RNA-seq analysis to study the macrophage response to different intracellular states of the model pathogen *Salmonella* enterica serovar Typhimurium (**Figure 1a and b**). The transcriptomes of individual infected macrophages revealed a spectrum of functional host response states to growing and non-growing bacteria. Intriguingly, macrophages harboring non-growing *Salmonella* display hallmarks of the proinflammatory M1 polarization state and differ little from bystander cells, suggesting that non-growing bacteria evade recognition by intracellular immune receptors (**Figure 1c**).
By contrast, macrophages containing growing bacteria have turned into an anti-inflammatory, M2-like state, as if fast-growing intracellular *Salmonella* overcome host defense by reprogramming macrophage polarization (**Figure 1c**). Additionally, our clustering approach reveals intermediate host functional states between these extremes. Altogether our data suggest that gene expression variability in infected host cells shapes different cellular environments, some of which may favor a growth arrest of *Salmonella* facilitating immune evasion and the establishment of a long-term niche; while others allow *Salmonella* to escape intracellular antimicrobial activity and proliferate.

Figure 1: Experimental strategy and result summary
a, Schematic representation of the workflow. Macrophages are infected with the intracellular pathogen *Salmonella* Typhimurium strain SL1344 harboring a dual-color reporter that comprises a constitutively (mCherry - red) and an arabinose-inducible fluorescent protein (GFP - green). 20 h after uptake, a heterogeneous population of host cells is detectable consisting of macrophages with growing bacteria (left), macrophages with non-growing bacteria (middle) and uninfected bystanders (right). Fluorescence-activated cell sorting (FACS) coupled to single-cell RNA-seq are used to sort and analyze these different subpopulations. **b,** Differential bacterial contents were checked using fluorescence microscopy and two representative images are shown (images on the right; Hoechst (blue), GFP (green), mCherry (red)). The white arrowhead indicates a non-growing *Salmonella* cell (yellow). Scale bar: 10 µm. **c,** Working model depicting the correlation between different macrophage activation programs and differentially growing *Salmonella*. Two distinct functional populations were found: a proinflammatory activation state dominated in macrophages with non-growing *Salmonella* and bystander cells, whereas an anti-inflammatory (M2-like) state prevailed in macrophages with growing bacteria.

Figure 1



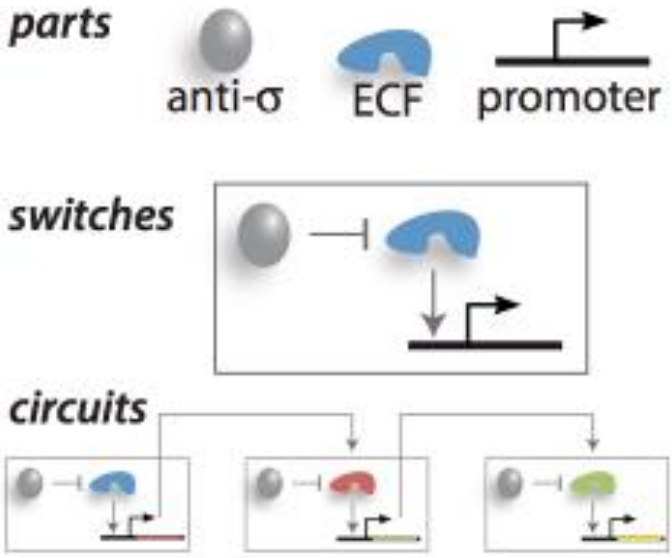
Presentation: Wednesday, 8 March 2017 from 14:45 - 15:00 in the Barbarossa Hall.

SHORT LECTURE 42
Synthetic Biology - Models, Tools, Applications (SL SMB)
08 March 2017 • 13:30 – 15:00

255/SMBV
Engineering orthogonal synthetic timer circuits in bacteria
S. Vecchione[1], D. Pinto[2], H. Wu[1], M. Mauri[1], T. Mascher[2], G. Fritz*[1]
[1]*LOEWE Center for Synthetic Microbiology, Marburg, Germany*
[2]*TU Dresden, Dresden, Germany*

The rational design of synthetic circuits is often restricted by cross-reactions between circuit components and physiological processes within the heterologous host. Here we present a strategy to overcome these restrictions by using extracytoplasmic function σ factors (ECFs). ECFs are not only the largest group of alternative sigma factors in bacteria, but also represent ideal orthogonal regulators because there exist over 90 phylogenetic ECF groups recognizing distinct target promoters. To explore the potential of ECFs for synthetic circuit design, we evaluate several heterologous ECFs in two phylogenetically diverse organisms - *Escherichia coli* and *Bacillus subtilis*. After a quantitative study of simple ECF switches, we use a computational modelling approach to predict the function of more complex ECF circuits. Specifically, we quantitatively predict the behaviour of a cascade with two and three ECFs, which we find in excellent agreement with experimental data. We show that in both organisms these "autonomous timer circuits" sequentially activate a series of target genes with a defined time delay. These results not only serve as a proof of concept for the application of ECF sigma factors as universal, organism-independent building blocks in synthetic biology, but could also be used, e.g. to introduce a proper timing hierarchy among the expression of biosynthetic pathway components in biotechnological applications.

Figure 1



Presentation: Wednesday, 8 March 2017 from 13:30 - 13:45 in room 13.

256/SMBV

A versatile dFBA simulator for modeling microbial communities

F. Centler*[1], F. Bonk[1], D. Taraba[1], D. Popp[1]
[1]*UFZ - Helmholtz Centre for Environmental Research GmbH, Department of Environmental Microbiology, Leipzig, Germany*

Introduction: Microbial organisms seldom occur in isolation but typically interact in communities of high diversity. The coexistence of microbes with different metabolic capacities allows them to perform complex tasks, from driving global elemental cycles to processing food and making nutrients available to their host in the human gut. For mono-clonal populations, Flux-Balance-Analysis (FBA) has been established as a standard modeling tool, allowing for quantitative predictions of metabolite turnover based solely on structural information of the intracellular metabolic network, linking genotype to phenotype. Its restriction to steady-state analysis has been overcome by dynamic FBA (dFBA) such that growth dynamics can be followed over time. We here present a formal extension of this modeling technique to microbial communities.
Methods: FBA models of different species are linked together, resulting in a community model. Community dynamics can be simulated over time in both batch and chemostat conditions. In the simulator, first metabolites need to be defined being present in the shared medium of the community. Then, species-specific exchange fluxes are linked to these medium metabolites, allowing for the exchange of these metabolites between community members. In each time step of the simulator, an FBA simulation is performed for each community member separately. Medium metabolite concentrations are then updated according to cumulated microbial uptake and secretion rates. The simulator is implemented in Matlab and can use both CellNetAnalyzer and the COBRA Toolbox for executing individual FBA simulations.
Results: We present the mathematical foundations and their implementation in a flexible and easy to use Matlab simulator code for dFBA-based community modeling. For demonstration purposes, we simulate a syntrophic relationship between the propionate oxidizing bacterium *Syntrophobacter fumaroxidans* and the methanogenic archaeon *Methanospirillum hungatei*.
Conclusion: The presented simulator can be used to study any microbial community consisting of species for which FBA models are available. Just as FBA-based approaches have been used successfully in metabolic engineering applications for strain optimization, we expect FBA-based community modeling to be elemental not only for the understanding of microbial community dynamics, but also for the de-novo design of systems with desired metabolic conversion capabilities within synthetic microbiology.

Presentation: Wednesday, 8 March 2017 from 13:45 - 14:00 in room 13.

257/SMBV

Towards a blank synthetic secondary chromosome: Characterisation and optimisation of synVicII in *Escherichia coli*

S. Messerschmidt [1], D. Schindler [1], C. Zumkeller *[1], F. Kemter [1], N. Schallopp [1], T. Waldminghaus[1]
[1]*Philipps-Universität Marburg, Synmikro, Marburg, Germany*

Introduction: Learning by building is one of the core ideas of synthetic biology research. Decreasing DNA synthesis costs and remarkable advancements of DNA assembly methods now allow answering research questions of bacterial chromosome biology. One approach of applying such synthetic genomics is the design, construction and analysis of secondary chromosomes. We previously introduced synVicII, a synthetic secondary chromosome in *Escherichia coli* [1]. Replication of synVicII is based on the replication mechanism of chromosome II in *Vibrio cholerae*.
Objectives: A deep understanding is a prerequisite for working with any genetic system. To this end, we characterised synVicII regarding its genetic integrity. Secondly, the handling of synVicII was optimised to establish a secondary chromosome backbone allowing fast and efficient assembly of larger replicons.
Methods: (A) Genome Integrity of synVicII was assessed by Southern Blot analysis and compared to an *oriC*-based minichromosome. (B) A directed evolution approach was performed to select for more stable variants of synVicII. (C) Different DNA cloning methods were used to obtain a conjugative synVicII with full compatibility to the popular Molecular Cloning (MoClo) assembly system. The MoClo compatible synVicII was used as Backbone for step-by-step MoClo assembly of the 100kb synVicII-NoMo.
Results: (A) Genome integrity of synVicII was found to be superior to that of an *oriC*-based secondary chromosome. (B) More stable variants of synVicII were selected and respective mutations raise interesting new questions about the synVicII replication mechanism. (C) Handling of synVicII was extensively improved, as demonstrated by the simple and fast assembly of the 100kb synVicII-NoMo. SynVicII-NoMo consists mostly of fully synthetic sequences lacking all known DNA motifs involved in chromosome maintenance. It will serve as blank template for future chromosome studies investigating the role of DNA motif distribution in chromosome maintenance.
Conclusion: The presented work confirms the suitability of synVicII as secondary chromosome system to study basic chromosome biology of bacteria. Likewise, thorough characterisation and optimisation of synVicII shown here, creates the basis for future applications of this secondary chromosome in the biotechnology industry.

References

[1] Messerschmidt, S. J., Kemter, F. S., Schindler, D. & Waldminghaus, T. Synthetic secondary chromosomes in *Escherichia coli* based on the replication origin of chromosome II in *Vibrio cholerae*. *Biotechnol. J.* 10, 302–314 (2015).

Presentation: Wednesday, 8 March 2017 from 14:00 - 14:15 in room 13.

258/SMBV

Analysis of protein secretion and thermophilic organisms – an expansion of the fluorescent protein toolbox

A. Woop*[1], M. Wingen[1], A. Heck[2], K. E. Jaeger[1,2], T. Drepper[1]
[1]*Heinrich Heine University, Institute of Molecular Enzyme Technology, Jülich, Germany*
[2]*Forschungszentrum Jülich, Institute of Bio- and Geosciences IBG-1, Jülich, Germany*

Fluorescent proteins such as the green fluorescent protein GFP have been extensively used as reporters in molecular biology, because they enable the *in vivo* analysis and visualization of complex cellular processes such as gene expression, protein localization or protein-protein interaction. However, the functionality of GFP and its homologues can be impaired by elevated temperatures, acidic pH, secretion *via* the general secretory (sec) pathway and especially the absence of molecular oxygen[1]. Therefore, a new class of flavin-binding fluorescent proteins (FbFPs and iLOV) have been developed, which were engineered from Light Oxygen Voltage (LOV) photoreceptor domains found in bacteria and plants[2,3]. In contrast to GFP, these LOV-based fluorescent proteins are relatively small and the formation and brightness of their fluorescence signal is not affected by low oxygen tension or pH. Here, we present the development of new, thermostable FbFP variants for the use in thermophilic bacteria as well as a novel, FbFP-based reporter system that allows the *in vivo* analysis of protein secretion *via* the sec-pathway.

LOV genes from genomes of various thermophilic bacteria and metagenomic libraries of hot springs in the Yellowstone National Park were used for the engineering of novel FbFP variants. Heterologous expression in *E. coli* yielded seven functional fluorescent proteins and the thermo-tolerance was subsequently characterized *in vitro*. We could identify two thermostable FbFPs exhibiting melting temperatures above 75 °C[4]. Furthermore, we constructed and evaluated new FbFP derivatives as reporters for the *in vivo* analysis of bacterial secretion processes. First, comparative secretion studies in *E. coli* revealed that in contrast to YFP, FbFPs can be translocated into the periplasm *via* the sec pathway in an active form. In order to create an FbFP-based secretion sensor, we used an FbFP that can bind Roseoflavin mononucleotide (RoFMN) which results in a red-shifted fluorescence in comparison to FMN. Since in *E. coli* flavins can only pass through the outer membrane, but not the inner one, addition of RoFMN to the medium allows to distinguish between secreted FbFP exhibiting red-shifted fluorescence and cytoplasmic protein that shows the typical cyan-green fluorescence.

Conclusion: The development of novel thermostable FbFPs and an FbFP-based secretion biosensor expands the toolbox of already available FbFP-based reporters and biosensors. This toolbox allows the *in vivo* investigation of important cellular processes and thereby provides important information for the optimization of biotechnological production processes.

References

[1] Drepper, T., Gensch, T. & Pohl, M. *Photochem. Photobiol. Sci.* 12, 1125–1134 (2013).
[2] Drepper, T. *et al.*. *Nat Biotech* 25, 443–445 (2007).
[3] Chapman, S. *et al.*. *Proc. Natl. Acad. Sci. U. S. A.* 105, 20038–43 (2008).
[4] Wingen, M. *Photochem. Photobiol. Sci.* accepted, (2016).

Presentation: Wednesday, 8 March 2017 from 14:15 - 14:30 in room 13.

259/SMBV

BEAP profile – a new and rapid test system for microbial status analysis and early detection of process incidents in agricultural biogas plants

S. Refai*[1], U. Deppenmeier[1]
[1]*University of Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*

Biogas is one of the most important renewable energy source and is used to produce electric power and heat, or it is purified to biomethane and fed into the national gas grid. The biogas production process in agricultural biogas plants is carried out with silaged material from renewable energy crops as well as with solid and liquid manure. The degradation of these organic materials is performed by a complex microbial community. There are four key metabolic processes (hydrolysis, acidogenesis, acetogenesis and methanogenesis) degrading the substrates into biogas which mainly consists of CO_2 and CH_4.

The biogas quantity, quality and the efficiency of the microbial degradation depend on the composition and the amount of the used substrates. Additionally, physico-chemical parameters such as pH, temperature, NH_4^+-N concentration, hydrogen carbonate buffer capacity and volatile fatty acid concentrations play a crucial role in biogas formation. The anaerobic conversion of organic material is usually monitored by physico-chemical parameters. However, it is not possible to analyze the overall performance of the microorganisms involved in the different digestion levels. A test system for the quantification of the metabolic capacity is not available. Consequently, it is difficult to determine the metabolic bottleneck during biogas production. Additionally, the early detection of imbalances in the microbial degradation process is challenging.

In this study, a system is described allowing to quantify the performance of microorganisms involved in different digestion levels in biogas plants. The test system (BEAP profile) is based on the addition of intermediates of the anaerobic degradation process, butyrate, ethanol, acetate, or propionate, to biogas sludge samples and subsequent analysis of CH_4 formation in comparison to control samples without supplementation within 24 h. The BEAP profile enables to monitor the metabolic capacity of the four main microbial degradation levels. Thus, it is possible to identify the rate-limiting step in biogas formation and to target beginning incidents in full-scale biogas plants.

More than 50 agricultural biogas plants were analyzed to distinguish between specific BEAP profiles for different types of process imbalances such as the beginning of NH_4^+-N intoxication, the start of acidification, insufficient hydrolysis, and potential mycotoxin effects. Additionally, the BEAP profile functions as a warning system to predict critical NH_4^+-N concentration thresholds leading to a drop of CH_4 formation and a sustainable process failure. Thus, the BEAP profiles enable to identify process limitations with respect to microbial degradation levels and to avoid process incidents in biogas plants.

Presentation: Wednesday, 8 March 2017 from 14:30 - 14:45 in room 13.

260/SMBV

Genetic engineering of *Oligotropha carboxidovorans* – a promising candidate for the aerobic utilization of industrial waste gases

D. Siebert*[1], A. Sage[1], V. Tschiginewa[1], B. Eikmanns[1]
[1]*Universität Ulm, Institut für Mikrobiologie und Biotechnologie , Ulm, Germany*

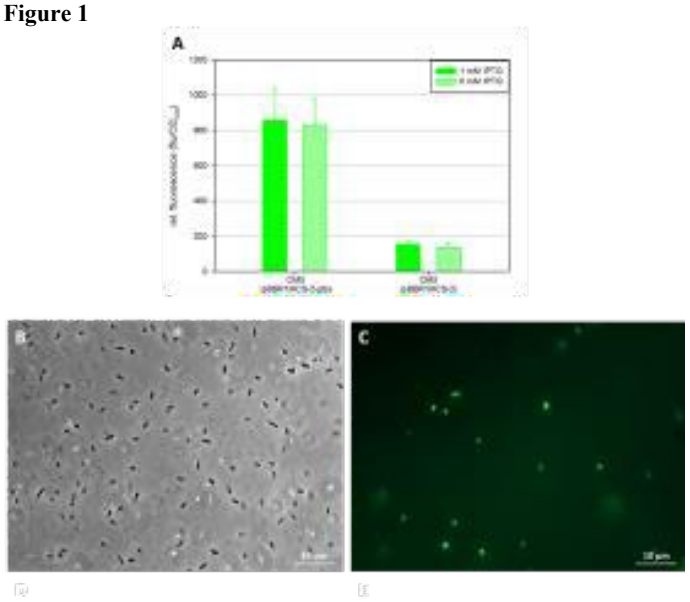
Introduction: Extensive research is carried out towards fermentation substrates which can be obtained from waste materials and are not competing with human nutrition. A promising technology platform is the fermentation of C1 gases which occur in industrial waste gases like syngas (mainly CO, CO_2 and H_2). While the anaerobic utilization of syngas is well-studied especially using *Clostridium* species, there is lacking knowledge for the aerobic utilization. The big advantage of an aerobic CO oxidation is the increased energy output for products with higher energy demand (Dürre & Eikmanns, 2015).
One promising candidate for aerobic (syn)gas fermentation is the autolithotrophic and carboxydotrophic *α-Proteobacterium Oligotropha carboxidovorans* strain OM5. This organism is characterized by its ability to utilize CO, CO_2 and H_2 as carbon and energy sources under aerobic conditions (Meyer & Schlegel, 1978). While in the past most of the available studies were focused on its oxygen-insensitive CO dehydrogenase, no approaches have been published for developing genetic tools for this organism.
Methods and Results: We were able to establish methods for genetic engineering of *O. carboxydovorans* OM5. First of all, it was possible to transform this organism with the broad-host-range vector pBBR1MCS-2 (Kovach *et al.*, 1995) and the newly constructed derivative pOCEx1 which allows inducible expression of inserted genes. The transformation was realized by applying a common electroporation procedure. Strains harboring one or the other vector were able to grow in minimal medium with yeast extract and acetate supplemented with kanamycin as selection marker. Extraction of the recombinant plasmids could be achieved using commercially available kit systems. Subsequently, the aim was the expression of a heterologous gene cloned into the plasmids using the gene encoding the green fluorescent protein (GFP). We detected the fluorescence of cultures with *O. carboxydovorans* OM5(pBBR1MCS-2-*gfp*) and were able to visualize this fluorescence under the microscope (Figure 1).
Conclusions: The expression of heterologous proteins with *O. carboxydovorans* OM5 is now available. This knowledge can be used to produce desired products via metabolic engineering using syngas under aerobic conditions.

References

[1] Dürre P, Eikmanns BJ: *Curr. Opin. Biotechnol.* 2015, 35:63-72
[2] Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM 2nd, Peterson KM: *Gene* 1995. 166(1):175-6
[3] Meyer O, Schlegel HG: *Arch. Microbiol.* 1978, 118(1):35-43

Figure 1: (A) Fluorescence measurement with OM5(pBBR1MCS-2-*gfp*) and (pBBR1MCS-2) cultivated with or without IPTG applying 475 nm excitation and 511 nm emission wavelength and normalized by the optical density (OD_{600}). Pictures of (B) light microscopy and (C) fluorescence microscopy at an emission wavelength of 509 nm with OM5(pBBR1MCS-2-*gfp*) cultivated in medium with IPTG.

Figure 1



Presentation: Wednesday, 8 March 2017 from 14:45 - 15:00 in room 13.

SHORT LECTURE 43
Diversity of bacterial gene regulation and signal transduction (SL GR)
08 March 2017 • 13:30 – 15:00

261/GRV

Cyclic di-nucleotide signalling in bacterial differentiation and antibiotic production

N. Tschowri*[1], M. A. Schumacher[2], R. G. Brennan[2], S. A. Neumann[1], M. J. Buttner[1,3]
[1]*Humboldt-Universität zu Berlin, Mikrobiologie, Berlin, Germany*
[2]*Duke University School of Medicine, Biochemistry, Durham, North Carolina, United States*
[3]*John Innes Centre, Molecular Microbiology, Norwich, United Kingdom*

The multi-talented bacteria *Streptomyces* have been awarded the Nobel Prize twice (1952 and 2015) for their exceptional ability to produce diverse medically-useful natural products. The synthesis of these secondary metabolites is genetically and temporally tightly interlinked with the developmental life cycle of Streptomycetes. Facing the urgent need for new antibiotics it is of particular significance to understand the signals and pathways that control development and thus antibiotic synthesis in these bacteria.
In our recent study, we have shown that the bacterial second messenger cyclic di-GMP (c-di-GMP), which is produced by GGDEF-type diguanylate cyclases and degraded by EAL or HD-GYP-type phosphodiesterases, determines the timing of differentiation initiation in *S. venezuelae* by regulating the activity of the highly conserved developmental master regulator BldD. Our structural and biochemical analyses revealed that a tetrameric form of c-di-GMP activates BldD DNA-binding by driving a unique form of protein dimerisation, leading to repression of the BldD regulon of sporulation genes during vegetative growth (1, 2, 3).
Currently, we aim to understand which of the 10 putative c-di-GMP-metabolising enzymes encoded by *S. venezuelae* contribute to c-di-GMP pool(s) sensed by BldD and how the BldD-c-di-GMP complex is assembled. Our initial data indicate that a distinct set of GGDEF / EAL proteins influences the developmental programme progression in *S. venezuelae* and that loading of BldD with tetrameric c-di-GMP is a two-step process.

Altogether, our work will greatly improve our understanding of *Streptomyces* physiology and c-di-GMP signalling in multicellular differentiation and secondary metabolite production and can contribute to a better exploitation of genetic engineering in *Streptomyces* for the production of antibiotics.

References

[1] Tschowri N, Schumacher MA, Schlimpert S, Chinnam NB, Findlay KC, Brennan RG, Buttner MJ. (2014) Tetrameric c-di-GMP Mediates Effective Transcription Factor Dimerization to Control Streptomyces Development. Cell, 2014 Aug 28;158(5):1136-47

[2] Bush MJ, Tschowri N, Schlimpert S, Flärdh K, Buttner MJ. c-di-GMP signalling and the regulation of developmental transitions in streptomycetes. (2015) Review. Nature Reviews Microbiology, 2015 Dec; 13(12):749-60

[3] Tschowri N. (2016) Cyclic dinucleotide-controlled regulatory pathways in Streptomyces. Review. Journal of Bacteriology, 2016 Jan;198 (1):47-54

Presentation: Wednesday, 8 March 2017 from 13:30 - 13:45 in room 10-11.

262/GRV

FnrL and three Dnr regulators control the anaerobic adaptation in *Dinoroseobacter shibae* DFL 12T

M. Ebert*[1], S. Laaß[2], D. Eckweiler[1], A. Thürmer[3], R. Daniel[3], E. Härtig[1], D. Jahn[1]

[1]*Technische Universität Braunschweig, Institute for Microbiology, Braunschweig, Germany*

[2]*Goethe-University Frankfurt, Institute for Molecular Biosciences, Frankfurt, Germany*

[3]*Göttingen Genomics Laboratory, Institute for Microbiology and Genetics, Göttingen, Germany*

Introducion: The marine bacterium *Dinoroseobacter shibae* DFL12T studied as a model organism of the Roseobacter group is able to adapt to low oxygen tension by using nitrate respiration and denitrification. The genome of *D. shibae* DFL12T comprises seven genes encoding members of the Crp/ Fnr family of transcriptional regulators. Members of this family are able to mediate metabolic adaptation with respect to a broad spectrum of intracellular and exogenous signals.

Objectives: We analyzed the role of the four regulators FnrL, DnrD, DnrF and DnrE of *D. shibae* in regulating genes encoding nitrate respiration and denitrification pathways. Moreover, we studied low oxygen tension and NO as signals for anaerobic gene expression.

Material & Methods: Knockout mutant strains for *fnrL*, *dnrD*, *dnrF* and *dnrE* of *D. shibae* were generated. We defined the regulons by comparing transcript levels of the regulatory mutant strains with the *D. shibae* wild type strain after shift from aerobic to anaerobic growth conditions. After regulon definition, we deduced specific binding sites for FnrL, DnrD, DnrF and DnrE by comparing the promoter regions of the target genes. Furthermore, we created promoter-*lacZ* reporter gene fusions of target genes to analyse the anaerobic expression in more detail. FnrL was produced in *E. coli* and after purification we performed spectroscopic analysis.

Results: Global sequence alignments were performed and grouped FnrL of *D. shibae* DFL12T to a *Roseobacter* specific phylum of FnrN type regulators of Crp/ Fnr transcriptional regulators. UV/ Vis spectroscopy of the purified FnrL protein revealed binding of an oxygen sensitive Fe-S cluster which enables Fnr to measure oxygen tension and regulate anaerobic gene expression. DnrD, DnrF and DnrE were classified within the Dnr phylum of Crp/ Fnr regulators. In *D. shibae* DFL12T the denitrification genes were found clustered and transcribed in large transcriptional units. The *napFDAGHBC* operon, encoding the nitrate reduction step, is regulated in a close interplay of all four Crp/ Fnr regulators of *D. shibae*. The *nirSECFDGHJN* encoding the nitrite reductase and the *norCBQDEF* operon encoding nitric oxide reductase were transcribed divergently sharing an overlapping promoter region. Both operons were activated by FnrL in an oxygen dependent manner and by DnrD in response to nitric oxide. Using promoter-*lacZ* reporter gene fusions the regulation by FnrL and DnrD was analysed in detail. Based on the defined regulons of the analysed transcriptional regulators a hierarchical network for anaerobic regulation of gene expression in *D. shibae* was established.

Conclusion: We determined the role of FnrL, DnrD, DnrF and DnrE regulators for the metabolic adaptation to low oxygen tension in *D. shibae*. Regulator specific binding motives were deduced and correlated with the condition dependent expression patterns. Moreover, a regulatory network was established.

Presentation: Wednesday, 8 March 2017 from 13:45 - 14:00 in room 10-11.

263/GRV

New insights into the general stress response of Bacillus subtilis – the SigB modulon.

A. Reder*[1], U. Mäder[1], U. Völker[1], U. Gerth[2], M. Hecker[2]

[1]*Universitätsmedizin Greifswald, Institut für Genetik - Abteilung Funktionelle Genomforschung, Greifswald, Germany*

[2]*Ernst-Moritz-Arndt Universität, Instutut für Mikrobiologie, Greifswald, Germany*

Almost all living cells have evolved a so called general stress response that is initiated in response to diverse stress signals in order to mediate increased resistance to a broad spectrum of otherwise lethal environmental stimuli. In *B. subtilis* the general stress response is under primary control of the alternative sigma factor SigB. Interestingly, activation of SigB by different stress stimuli does not cause identical induction ratios of all SigB regulated genes, but rather causes a finely tuned target gene expression level that is specific to the features of the initial stress stimulus, indicating downstream signal integration beyond the primary decision of SigB activation. In addition to transcriptional activation by SigB, it was striking that many genes also seem to be repressed in a strictly SigB dependent but still unknown fashion. To address these and other questions dealing with the size and structure of the SigB regulon as well as the underlying regulatory mechanisms we performed global transcriptomics and proteomics experiments on the *B. subtilis* wild-type 168 compared to a set of mutant strains including the Δ*sigB*, Δ*mgsR*, Δ*spx*-mutants and the Δ*mgsR*/Δ*spx* double mutant as well as a mutant allowing xylose dependent expression of SigB (P_{xylA}::*sigB*). The data thus obtained show that the SigB regulon is much larger and that its structure is much more complex than expected. For example secondary regulators such as Spx and MgsR come into play to integrate secondary signals beyond the primary decision of SigB activation creating stress specific sub-regulons. Furthermore, we can demonstrate that SigB is also responsible to coordinately shut off the expression of hundreds of genes in face of harsh environmental stress. Among many others two major examples for this observation are the SigB dependent inactivation of the master regulator of sporulation (Spo0A) as well as competence development (ComK). Here we would like to present an elaborate model of a hierarchically ordered regulatory pathway with SigB as the primary master regulator functioning as a transcriptional activator that allow the downstream-integration of secondary stress stimuli for a finely tuned target gene expression and possessing a new role as global emergency brake system to silence alternative developmental programs whose expression would be inappropriate under severe SigB inducing stress conditions.

Presentation: Wednesday, 8 March 2017 from 14:00 - 14:15 in room 10-11.

264/GRV

Analysis of quinone mutants in respect to ArcA phosphorylation and product formation

A. Nitzschke*[1], K. Bettenbrock[1]
[1] *MPI for dynamics of complex technical systems, Magdeburg, Germany*

E.coli is able to respond to changes in the oxygen availability through regulation of its metabolism. Two major transcription factors (TF) are responsible for this adaption, the two-component system ArcB/A und the global TF FNR. In contrast to FNR, the two-component system ArcB/A reacts only indirectly to the change in oxygen supply. Rather other signals seem to have an influence on the ArcB/A activation. The effect of the redox pool of the cell on the activation of ArcA has already been discussed in literature, whereby the focus has been on the redox ratio of the quinones (1). Quinones function as electron carriers between the dehydrogenases and oxidases of the electron transport chain (ETC). *E.coli* possesses three different quinone species: ubiquinone (UQ), which is synthesized mainly during aerobic conditions and demethylmenaquinone (DMK) und menaquinone (MK), which are synthesized mainly during anaerobic respiration (2).
To study in more detail the function of different quinone species, strains with deletions preventing UQ synthesis, as well as MK and/or DMK synthesis were cultured under aerobic as well as anaerobic conditions. The data clearly show an influence of the different quinones on the production of fermentation products both at aerobic but also anaerobic conditions. A special focus was on deriving a correlation between the compositions of the quinone pool und the ArcA phosphorylation state. In contrast to the indirect measurements of ArcA phosphorylation by reporter genes, we determined the relative phosphorylation state of the TF directly by Phos-tag SDS-PAGE and Western Blotting. The results from the characterization of the mutants compared to the wild type strain MG1655 showed that in contrast to the in vitro results (3), in vivo, no inhibitory effect from the UQ on the ArcA phosphorylation was observed.
Furthermore gene expression analysis under aerobic conditions showed that the ubiquinone knockouts exhibit a "pseudo" fermentative state independent of the phosphorylation of ArcA. This indicates that it is problematic and error-prone to deduce the phosphorylation state of ArcA from indirect measurements using reporter gene fusions and might also hint to additional factors determining ArcA activity. A comprehensive analysis of gene expression in the three quinone deletion strains under anaerobic conditions is currently performed to further elucidate the function of quinones at fermentative conditions.

References

[1] Malpica, R. and Franco, B. (2004) Identification of a quinone-sensitive redox switch in the ArcB sensor kinase (2004), Proc. Natl. Acad. Sci. USA 101(36), 13318-13323.
[2] Bekker M. (2009), Respiratory electron transfer in Escherichia coli: components, energetics and regulation (2009).
[3] Georgellis D. and Kwon O. (2001), Quinones as the redox signal for the arc two-component system of bacteria (2001), Science 292(5525), 2314-2316.

Presentation: Wednesday, 8 March 2017 from 14:15 - 14:30 in room 10-11.

265/GRV

Transcription factors TFE, Spt4/5 and the archaeo-viral factor ORF145 regulate the archaeal RNA polymerase by modulating the conformation of the mobile clamp domain

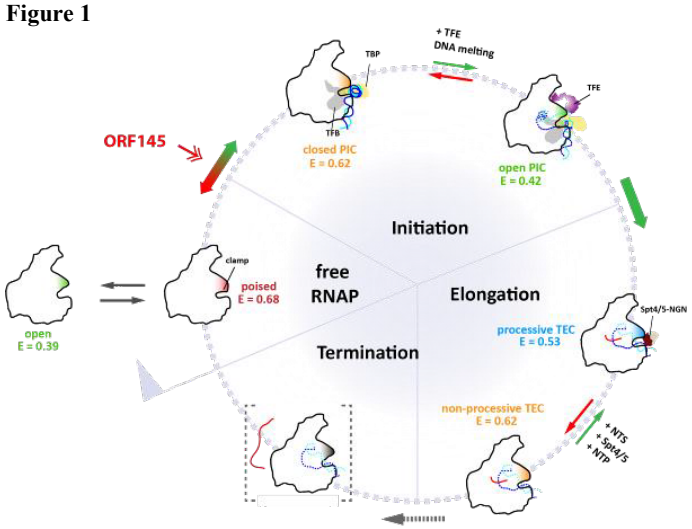
S. Schulz[1], C. Sheppard[2], P. Tinnefeld[1], F. Werner[2], D. Grohmann*[1,3]
[1]*Institut für Physikalische und Theoretische Chemie, NanoBioSciences, Braunschweig, Germany*
[2]*University College London, Department of Structural and Molecular Biology, London, United Kingdom*
[3]*Universität Regensburg, Lehrstuhl für Mikrobiologie, Regensburg, Germany*

Transcription is an intrinsically dynamic process and requires the coordinated interplay of RNA polymerases (RNAPs) with nucleic acids and transcription factors (1). Classical structural biology techniques have revealed detailed snapshots of a subset of conformational states of the RNAP as they exist in crystals. A detailed view of the conformational space sampled by the RNAP and the molecular mechanisms of the basal transcription factors TFE and Spt4/5 through conformational constraints has remained elusive. We have monitored the conformational changes of the flexible clamp of the RNAP by combining a fluorescently labeled recombinant 12-subunit RNAP system with single-molecule Förster resonance energy transfer (FRET) measurements. We measured and compared the distances across the DNA binding channel of the archaeal RNAP and correlated these results to functional data (2). Our results show that the transition of the closed to the open initiation complex, which occurs concomitant with DNA melting, is coordinated with an opening of the RNAP clamp that is stimulated by TFE. We show that the clamp in elongation complexes is modulated by the non-template strand and by the processivity factor Spt4/5, both of which stimulate transcription processivity. We furthermore demonstrate that the newly discovered viral factor ORF145 targets the clamp, locks it in one defined conformation and thereby represses transcription (3). Taken together our results reveal an intricate network of interactions within transcription complexes between RNAP, transcription factors and nucleic acids that allosterically modulate the RNAP during the transcription cycle.

References

[1] Werner F. and Grohmann D. (2011). Evolution of multisubunit RNA polymerases in the three domains of life. Nature Reviews Microbiol.9:85-98
[2] Schulz S., Gietl A., Smollett K., Tinnefeld P., Werner F. and Grohmann D. (2016). TFE and Spt4/5 open and close the RNA polymerase clamp during the transcription cycle. PNAS, 113:E1816-25.
[3] Sheppard C., Blombach F., Belsom A., Schulz S., Daviter T., Smollett K., Mahieu E., Erdmann S., Tinnefeld P., Garrett R., Grohmann D., Rappsilber J., Werner F. (2016) Repression of RNA polymerase by the archaeo-viral regulator ORF145/RIP. Nature Communications, 7:13595.

Figure 1



Presentation: Wednesday, 8 March 2017 from 14:30 - 14:45 in room 10-11.

266/GRV
Regulation of cyanobacterial phototaxis
A. Wilde*[1], A. Jakob[1], T. Wallner[1], N. Schuergers[1], C. Mullineaux[1]
[1]*University of Freiburg, Institute of Biology, Freiburg, Germany*

The cyanobacterium *Synechocystis* 6803 exhibits flagellar-independent twitching motility using type IV pili. Phototaxis is a mechanism that allows cyanobacteria to respond to fluctuations in the quality and quantity of illumination by moving either towards or away from a light source. Regulation of phototactic motility involves many different gene products, including various photoreceptors, second messengers and the RNA chaperone Hfq. Previously, we showed that individual *Synechocystis* sp. PCC 6803 cells do not respond to a spatiotemporal gradient in light intensity, instead they directly and accurately sense the position of a light source. Single cells of *Synechocystis* sp. PCC6803 focus the light from a unidirectional light source in a sharp focal point on the distal side of the cell (Schuergers et al., 2016). We were able to demonstrate that this focusing effect correlates with phototactic movement. However, the signal transduction pathway regulating the motility apparatus in a polar light-dependent manner remains elusive. We propose a model where the strong focal point induces a local inhibition of the motility apparatus at the shadow-side surface of the cell. At least 3 operons encode signaling systems that are possibly involved in the regulation of phototaxis and show similarity to known classical chemotaxis regulators. Using fluorescence microscopy we determined the localization of CheY-like and PATAN-domain response regulators and phototaxis photoreceptors. We show that the expression of these response regulators can regulate direction of movement. In addition, we show that ethylene interferes with an UV-A light photoreceptor, which controls synthesis of one of these response regulators leading to acceleration of motility.

References
[1] Schuergers N, Lenn T, Kampmann R, Meissner MV, Esteves T, Temerinac-Ott M, Korvink JG, Lowe AR, Mullineaux CW, Wilde A. (2016) Cyanobacteria use micro-optics to sense light direction. *eLife* 5 doi: 10.7554/eLife.12620.

Presentation: Wednesday, 8 March 2017 from 14:45 - 15:00 in room 10-11.

SHORT LECTURE 44
Diversification in the environment and the mammalian host (SL MDE)
08 March 2017 • 13:30 – 15:00

267/MDEV
Community composition of Bacteria and Archaea from hot marine hydrothermal vents of Vulcano Island
M. Suleiman*[1], C. Schäfers[1], K. O. Stetter[2], G. Antranikian[1]
[1]*Technical University of Hamburg, Institute of technical microbiology, Hamburg, Germany*
[2]*University of Regensburg, Regensburg, Germany*

Introduction: Hyperthermophilic microorganisms, with preferable growth temperatures between 80 °C – 100 °C, are inhabitants of marine and terrestrial hydrothermal vents and chimneys. Most studies have focused on the exploration of thermophilic communities with preferable growth temperatures between 50 °C and 70 °C. Therefore, the diversity of truly hyperthermophilic microorganisms of habitats around 100 °C are still poorly understood.
Objectives: For obtaining new insights in community composition of hyperthermophilic microorganisms, sediment- and water-samples were taken from two hydrothermal vents with temperatures of 100 °C at Vulcano Island, Italy. Our study goal was (i) to analyze the community composition and species abundance of two marine hydrothermal vents (100 °C) and (ii) to analyze the community composition of hyperthermophilic enrichment cultures growing heterotophically on carbohydrates.
Material and Methods: We used a combination of denaturent gradient gel electrophoresis (DGGE) and MiSeq metagenomic sequencing for microbial community analysis of the original samples in addition to the enrichment cultures. The enrichment cultures were cultivated with cellulose, locust bean gum and starch as substrates and incubation temperature of 90 °C.
Results and Conclusion: Our results showed a high abundance of hyperthermophilic Archaea, specially in one sample, and a similar diverse archaeal community composition in both samples. Specially the genera *Staphylothermus*, *Thermococcus* and strains of the aerobic hyperthermophilic genus *Aeropyrum* were abundant. Regarding the bacterial community, ε-proteobacteria, specially the genera of *Sulfurimonas* and *Sulfurovum*, were highly abundant and the chemolithoautrophic mesophilic species *Sulfurimonas autotrophica* seemed to dominate both habitats. The microbial diversity was significantly changed depending on the carbon source. An overview of the microbial community of two marine habitats and the enrichment culture will be presented.

Presentation: Wednesday, 8 March 2017 from 13:30 - 13:45 in room 5.

268/MDEV
Phylogeny and wide distribution of anoxygenic photosynthesis among Proteobacteria
J. F. Imhoff*[1], T. Rahn[1], S. Neulinger[2]
[1]*GEOMAR Helmholtz Center for Ocean Research, Marine Microbiology, Kiel, Germany*
[2]*omics2view.consulting GbR, Kiel, Germany*

Introduction: The ability to perform anoxygenic photosynthesis is widely distributed among eubacteria and found in different phyla of Chloroflexi, Chlorobi, Firmicutes, Acidobacteria and Proteobacteria, which employ distinctive photosystems for performing photosynthetic energy conversion. Traditionally the phototrophic purple bacteria are known as anaerobic bacteria, growing and performing photosynthesis under anoxic conditions. In addition, a large group of bacteria performing anoxygenic photosynthesis under oxic conditions is abundant and of importance in ocean waters. The phylogenetic identification of

established species is prerequisite to identify these bacteria in environmental metagenomes and to estimate the relative importance of individual species. For this purpose the *pufLM* genes coding for reaction center proteins of phototrophic purple bacteria were established as functional molecular marker.
Objectives: In order to retrace the phylogeny of the photosynthetic reaction center and of anoxygenic phototrophic purple bacteria and to establish a solid reference for environmental studies of these bacteria, gene sequences of 16S rRNA and *pufLM* gene were analyzed of more than 600 strains including all available type strain sequences.
Materials & methods: Sequences from *pufLM* and 16S rRNA genes of corresponding strains were retrieved from genome sequences, databases and newly determined from cultures by using established primer systems and sequencing technologies. Sequences were aligned against the SILVA database, trimmed to a common length with mothur and the alignments were improved by manual editing. Maximum likelihood phylogenetic trees were calculated with the program IQ-TREE.
Results: Ability to perform photosynthesis is deeply rooted within the Proteobacteria. Representatives of the anaerobic phototrophic purple bacteria form distinct lineages in Alpha-, Beta- and Gammaproteobacteria: in the orders Rhodospirillales, Rhizobiales and Rhodobacterales, in the Burkholderiales, Rhodocyclales and Chromatiales. As revealed by *pufLM* gene sequences, the aerobic phototrophic purple bacteria add to the phylogenetic diversity with groups represented by Cellvibrionales, Sphingomonadales and Caulobacterales. A comprehensive overview is presented on the 16S rRNA gene based phylogeny and compared to the phylogenetic relations of the *pufLM* genes of all available representative type strains. To the overwhelming part congruent phylogenies were observed with both approaches. In consequence, sequences of *pufLM* genes are perfect tools to study the diversity of phototrophic purple bacteria in environmental communities.
Conclusion: The results shed light on the phylogeny of *pufLM* and anoxygenic photosynthesis and have strong implications of studies on the diversity and ecological relevance of phototrophic bacteria.

Presentation: Wednesday, 8 March 2017 from 13:45 - 14:00 in room 5.

269/MDEV
Foreign affairs: Plasmid transfer between roseobacters and rhizobia
P. Bartling*[1,2], B. Bunk[1], J. Overmann[1], H. Brinkmann[1], J. Petersen[1]
[1]*DSMZ, Braunschweig, Germany*
[2]*DSMZ, Roseobacter, Braunschweig, Germany*

A multipartite genome organization with a chromosome and many extrachromosomal replicons (ECRs) is characteristic for *Alphaproteobacteria*. The probably best investigated ECRs of terrestrial rhizobia are symbiotic plasmids for legume root nodulation and the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens*. The great relevance of ECRs for the marine Roseobacter group (*Rhodobacteraceae*) is exemplified by the biofilm plasmid of *Phaeobacter inhibens* DSM17395. RepABC plasmids represent the most abundant alphaproteobacterial replicon type and phylogenetic analyses of the replication module showed a strict separation of rhizobia and *Rhodobacteraceae*. Their distinctness led to our working hypothesis of a plasmid-bacterial co-evolution that resulted in an order-specific host range. In the current study we investigated more than 1,400 rhizobial genomes and surprisingly identified two Roseobacter-specific RepABC-type operons located on a 259-kb plasmid of *Martelella mediterranea* DSM17316T, whose genome was completely sequenced with the PacBio-technology, and a 322-kb replicon of *Rhizobium* sp. NT-26. Both ECRs harbor a RepC-1 replicase and phylogenetic analyses of their replication modules document an independent origin. Both plasmids contain additional rhizobial RepABC modules and likely emerged from fusion events of a pre-existing rhizobial ECR with a conjugated equivalent from roseobacters. This prediction is validated by systematic HGT analyses, which revealed that up to 61% of their genes originated from *Rhodobacteraceae*, and by the presence of Roseobacter-specific type IV secretion systems. Functionality tests of the respective replication modules showed that all investigated RepABC operons are working. Furthermore, they documented that genuine rhizobial RepABC plasmids are only maintained in *A. tumefaciens*, but not in *P. inhibens*, which is in agreement with our initial working hypothesis. However, we surprisingly showed that the Roseobacter-like replication systems from *Martelella* and *Rhizobium* sp. NT-26 are functional in both host strains. Accordingly, we tested a complete set of Roseobacter RepABC-modules from all nine compatibility groups and documented that all operons except RepC-8 are replicated in *Agrobacterium* providing evidence for a broader host range of rhodobacteracean plasmids than expected. The current study revealed the presence of natural shuttle vectors that mediate the genetic exchange between roseobacters and rhizobia thus providing first insights into underlying mechanisms that result in the phylogenetic diagnosis HGT.

Presentation: Wednesday, 8 March 2017 from 14:00 - 14:15 in room 6.

270/MDEV
Microevolution of *Pseudomonas aeruginosa* in cystic fibrosis lungs
J. Klockgether*[1], S. Fischer[1], N. Cramer[1], L. Wiehlmann[1], B. Tümmler[1]
[1]*Hannover Medical School, Clin. Research Group - OE 6711; Ped. Pneumology, Hannover, Germany*

In the airways of most cystic fibrosis (CF) patients chronic infections with the opportunistic pathogen *Pseudomonas aeruginosa* are established during childhood and typically determine the clinical course. Over the years the bacteria undergo microevolution presumably enhancing the adaptation to the lung habitat.
At our local CF clinic, semiannual collection of *P. aeruginosa* isolates from 35 chronically infected patients was initiated in the 1980s. Microevolution was investigated for isolates from twelve patients, six with the mildest and six with the most severe clinical courses, in order to monitor microevolution against the background of the disease.
For these courses, genomes of sequential isolates of the initially colonizing clone were sequenced (approx. 170 isolates in total). Screens for single nucleotide polymorphisms (SNPs), small indels, larger deletions and accessory genome variations were done by de novo assembly of sequencing reads and alignments to a reference genome. The sequential isolates were also characterized in mutation rates and phenotypic traits such as morphology, motility and virulence effector secretion.
The microevolution within the twelve courses was monitored by constructing phylogenetic trees according to the chronological order of acquisition of unique and/or shared genome variations. We observed the whole spectrum of evolutionary modes ranging from the presence of a single adapted strain to the long-term persistence of co-existing clades with several mixed types in between.
Overall, >4800 mutations occurred in the twelve courses, mostly SNPs but also indels affecting one or two nucleotides. Loss of greater DNA blocks occurred rarely. Certain genes were found to be affected in several patient courses, often even by identical mutations. The majority of these CF lung microevolution hotpots were associated with either antimicrobial resistance or the biosynthesis of bacterial surface components such as alginate. For some courses, an increased occurrence of severe mutations (stop-

mutations, frameshifts) in key genes of lifestyle was detected, but descendants of the respective isolates were not recovered from the lungs later.
When comparing the results for courses from mildly and severely affected patients, hints for different *P. aeruginosa* microevolution in the respective airway habitats could be found. Stop mutations and frameshifts occurred more often in the isolates from severely affected patients, and the co-existence of persisting clades was less frequent for the severe courses.
Supported by the DFG (SFB900) and the Christiane Herzog Stiftung.

Presentation: Wednesday, 8 March 2017 from 14:15 - 14:30 in room 6.

271/MDEV
Evolution of mismatch repair-deficient *P. aeruginosa* under small population bottlenecks results in preferential accumulation of synonymous mutations
I. Grekov*[1], U. Muthukumarasamy[1], S. Häußler[1]
[1]*Helmholtz Centre for Infection Research, Molecular Bacteriology, Braunschweig, Germany*

Question: Mutation accumulation experiments are an invaluable tool for studying bacterial evolution. They are essential for the understanding of forces shaping genetic variation. Considering that the design of the majority of mutation accumulation experiments included weak or intermediate bottleneck intensity, we investigated bacterial evolution under extremely strong bottlenecking, which minimizes natural selection and maximizes genetic drift.
Methods: Six parallel populations of mismatch repair-deficient *P. aeruginosa* were propagated for ~ 956 generations. The populations were passed through 44 single-cell bottlenecks. Afterwards, we used whole genome sequencing to investigate mutation rate as well as types of accumulated mutations. We also compared the fitness of mutation accumulation lines in the beginning and in the end of the experiment.
Results: It was shown that the experimental populations accumulated on average 146 mutations, which is in agreement with the previous studies of mutation rate in mismatch repair-deficient bacteria (Heilbron *et al.*, 2014). The detailed analysis revealed that 49.15% of mutations were synonymous substitutions, 26.55% were non-synonymous substitutions, 4.53% were short insertions/deletions in coding regions and 11.88% were mutations in intergenic regions. Short insertions/deletions occurred in genes with generally lower expression. Finally, the comparison of fitness revealed that the populations evolved under strong bottlenecking had substantially lower growth rate in comparison with starting populations.
Conclusion: Under extremely strong bottlenecking, mismatch repair-deficient *P. aeruginosa* rapidly accumulated multiple mutations, which led to the loss of fitness. At the same time, sequencing results demonstrated an unusually high ratio of synonymous to non-synonymous mutations. This implies a possibility that, even under strong bottlenecking, there is substantial selection against potentially more harmful non-synonymous mutations.

References
[1] Heilbron K, Toll-Riera M, Kojadinovic M, MacLean RC. Fitness is strongly influenced by rare mutations of large effect in a microbial mutation accumulation experiment. Genetics. 2014;197(3):981-90.

Presentation: Wednesday, 8 March 2017 from 14:30 - 14:45 in room 7.

272/MDEV
The pig intestinal bacterial collection allows functional studies of gut microbiota-host interactions in colorectal cancer
D. Wylensek*[1], A. Afrizal[1], B. Fösel[2], B. Abt[2], S. Heinzmann[3], L. Gau[4], K. Wegner[4], I. Lagkouvardos[1], P. Schmitt-Kopplin[3], J. Schmid[5], K. Flisikowski[6], S. Rohn[4], J. Overmann[2], A. Schnieke[6], T. Clavel[1,6]
[1]*ZIEL - Zentralinstitut für Ernährungs- und Lebensmittelforschung, Core Facility Microbiom / NGS, Freising, Germany*
[2]*Leibniz-Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[3]*Helmholtz Zentrum München, Analytical BioGeoChemistry, Neuherberg, Germany*
[4]*University of Hamburg, Hamburg School of Food Science, Hamburg, Germany*
[5]*Technical University of Munich, Chair of Chemistry of Biogenic Resources, Straubing, Germany*
[6]*Technical University of Munich, Chair of Livestock Biotechnology, Freising, Germany*

The intestinal microbiota is a major environmental factor influencing human health and the development of chronic diseases such as colorectal cancer (CRC). Animal models are very helpful to investigate mechanisms underlying disease, but mouse models of CRC show limitations in terms of disease phenotype, gut physiology, and nutrition. Here we use a recent genetically-modified pig model for colonic tumorigenesis (*APC*1311+/- pigs) to gain novel insights into microbe-host interactions involved in CRC development.
To assess whether the gut microbial ecosystem is disturbed in *APC*1311+ vs. wildtype pigs, we monthly collected faecal samples from 17 pigs over the period of a year for characterization by high-throughput 16S rRNA gene amplicon sequencing. Preliminary data show divergence of fecal microbiota profiles between *APC*1311+ pigs and controls with increasing age. Dysbiosis at the level of metabolites is currently under investigation using NMR-based metabolomics. To assess bacterial functions in greater detail, we obtained isolates from different gut locations by means of anaerobic cultivation and intend to establish the first publically available collection of bacterial strains from the pig intestine. To date this collection contains approximately 100 species representing 32 different families from 10 phyla, including 19 strains proposed to represent novel taxa. Isolates were tested in vitro for their ability to metabolize bile acids, which have been implicated in CRC development but experimental evidence is lacking. So far, a total of 25 strains were positive for bile salt hydrolase, including phylogenetically diverse bacteria (e.g. *Bacteroides*, *Bifidobacterium*, *Enterococcus*, *Fusobacterium*, *Lactobacillus*, *Prevotella*, *Streptococcus* spp.). Moreover, one strain of the species *Clostridium scindens* was capable to produce secondary bile acids via dehydroxylation and dehydrogenation of the primary bile acids cholic acid.
Ongoing work combining feeding experiments and the establishment of minimal bacterial consortia in continuous culture will allow detailed investigation of bacterial functions of relevance in CRC.

Presentation: Wednesday, 8 March 2017 from 14:45 - 15:00 in room 7.

SHORT LECTURE 45
Electron transfer in metabolism (SL GMB / SL SM)
08 March 2017 • 13:30 – 15:00

273/GMBV

Acetone degradation in the sulfate-reducing bacterium *Desulfococcus biacutus*: solving an old mystery piece by piece

J. Frey*[1], D. Spiteller[1], T. Huhn[2], D. Schleheck[1], B. Schink[1]
[1]*University of Konstanz, Biology, Konstanz, Germany*
[2]*University of Konstanz, Chemistry, Konstanz, Germany*

The biochemistry of acetone degradation is well understood in aerobic and nitrate-reducing bacteria. These bacteria activate acetone via carboxylation to acetoacetate which consumes at least two ATP equivalents. For sulfate reducers, such an expensive activation reaction is hardly possible, due to their limited energy budget. Furthermore, acetoacetate was ruled out as an intermediate, and neither acetone carboxylase activity nor acetone carboxylases were found in the genome of *D. biacutus* (1,2).
Recent studies on the sulfate-reducing, acetone-utilizing bacterium *Desulfococcus biacutus* indicated an involvement of ATP-, TDP (thiamine diphosphate)- and B12-dependent enzymes, leading finally to acetoacetyl-CoA (3,4). Additionally, the sequenced genome of *D. biacutus* allowed comparative 2D-PAGE analysis leading to the identification of several induced proteins during growth with acetone, which are potentially involved in acetone degradation (2).
Several candidate enzymes (two dehydrogenases, a TDP-dependent enzyme and a B12-dependent mutase) were successfully cloned and overexpressed in *Escherichia coli*. Purified recombinant enzymes were used for further analysis via HPLC and LC-MS. One of these enzymes exhibited an aldehyde/ketone oxidoreductase activity, the mutase performed an isomerization of 2-hydroxyisobutyryl-CoA to 3-hydroxybutyryl-CoA and a dehydrogenase was capable of oxidizing 3-hydroxybutyryl-CoA to acetoacetyl-CoA (5). The initial activation step (which appears to involve an activated formyl residue) of acetone degradation by *D. biacutus*, is still under research and would represent the missing piece to finally resolve the puzzle of this novel biochemical pathway.

References

[1] Janssen, P. H., and Schink, B. (1995a) Catabolic and anabolic enzyme activities and energetics of acetone metabolism of the sulfate-reducing bacterium *Desulfococcus biacutus*. *Journal of bacteriology* 177, 277-282
[2] Gutiérrez Acosta, O. B., Schleheck, D., and Schink, B. (2014b) Acetone utilization by sulfate-reducing bacteria: draft genome sequence of *Desulfococcus biacutus* and a proteomic survey of acetone-inducible proteins.
[3] Gutiérrez Acosta, O. B., Hardt, N., and Schink, B. (2013) Carbonylation as a key reaction in anaerobic acetone activation by *Desulfococcus biacutus*. *Applied and environmental microbiology* 79, 6228-6235
[4] Gutiérrez Acosta, O. B., Hardt, N., Hacker, S. M., Strittmatter, T., Schink, B., and Marx, A. (2014a) Thiamine pyrophosphate stimulates acetone activation by *Desulfococcus biacutus* as monitored by a fluorogenic ATP analogue. *ACS chemical biology*
[5] Frey, J., Rusche, H., Schink, B., and Schleheck, D. (2016) Cloning, functional expression and characterization of a bifunctional 3-hydroxybutanal dehydrogenase /reductase involved in acetone metabolism by *Desulfococcus biacutus*. *BMC Microbiology* in press

Presentation: Wednesday, 8 March 2017 from 13:30 - 13:45 in room 12.

274/GMBV

Analysis of intracellular NADPH accumulation in *C. glutamicum* using the sensor-probe mBFP

O. Goldbeck*[1], D. Petrov[2], G. Seibold[1]
[1]*Universität Ulm, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany*
[2]*Universität zu Köln, Institut für Biochemie, Köln, Germany*

Question: *Corynebacterium glutamicum* is particularly known for its industrial application in the production of amino acids. Amino acid overproduction comes along with a high NADPH demand, which is covered mainly by the oxidative part of the pentose phosphate pathway (PPP). Complete redirection of the carbon flux toward the PPP by deletion of the *pgi* gene, encoding the phosphoglucoisomerase, has been applied for the improvement of *C. glutamicum* strains, but this was accompanied by severe negative effects on growth and sugar uptake. Inhibition of sugar uptake occurred in *C. glutamicum* Δ*pgi* within seconds, which might be indirectly triggered by high intracellular NADPH concentrations. However, kinetics of NADPH accumulation have not been investigated in bacteria so far, due to the lake of tools for fast, continuous online-analysis.
Methods: The metagenome derived SDR-family protein mBFP was shown to exhibit blue fluorescence upon NADPH binding1. The plasmid encoded, codon-usage optimized mBFP-variant mBFPopt was used to analyze NADPH generation kinetics in *C. glutamicum* strains with altered NADPH metabolism.
Results: The optimized sensor protein mBFPopt allows kinetic analyses of NADPH accumulation in *C. glutamicum* strains. By this means we observed in small scale, short time experiments a fast accumulation of NADPH in *C. glutamicum* Δ*pgi*. NADPH accumulation occurred in *C. glutamicum* Δ*pgi* within seconds after the addition of the carbon source glucose. NADP accumulation proceeded more slowly in *C. glutamicum* WT and *C. glutamicum* strains overexpressing genes for transhydrogenases. These results show the suitability of the method for analyses of intracellular NADPH accumulation kinetics. Based on the observed fast NADPH accumulation in *C. glutamicum* Δ*pgi* we propose the following order of events leading to the inhibition of sugar uptake. After glucose addition NADPH accumulates very fast. As the first enzyme of the PPP glucose-6-P-dehydrogenase is inhibited at excess NADPH2, the fast NADPH accumulation also leads to fast accumulation of Glc-6-P, which the leads to the inhibition of the sugar transporters.

References

[1] Hwang *et al*., 2012, Screening of a highly soluble and oxygen-independent blue fluorescent protein from metagenome. Biochem. Biophys. Res. Commun. 419, 676–681.
[2] Moritz *et al*., 2000, Kinetic properties of the glucose-6-phosphate and 6-phosphogluconate dehydrogenases from *Corynebacterium glutamicum* and their application for predicting pentose phosphate pathway flux *in vivo*. Eur. J. Biochem. 267:3442–3452

Presentation: Wednesday, 8 March 2017 from 13:45 - 14:00 in room 12.

275/GMBV

Electron transport chains without quinones: A multiprotein complex allows stand-alone respiration

K. Seidel*[1], L. Adrian[1], P. Walter[1]
[1]*UFZ - Helmholtz Centre for Environmental Research, Isotope Biogeochemistry, Leipzig, Germany*

Respiratory chains convert redox potential differences of redox pairs into metabolically accessible energy, mostly employing a series of redox active protein complexes linked with membrane soluble electron shuttle pools such as quinones. In *Dehalococcoides mccartyi* strain CBDB1 a novel modus was

hypothesized, where the protein complex for respiratory reductive dehalogenation does not require quinones or cytochromes by channeling electrons exclusively within this complex [1] and is hypothesized to thereby pump protons across the membrane. The complex contains a hydrogen uptake hydrogenase that oxidizes hydrogen, transfers electrons via iron-sulfur clusters to a yet-uncharacterized iron-sulfur cluster binding protein, and a complex iron-sulfur molybdoenzyme without known function onto a reductive dehalogenase (RdhA). The reductive dehalogenase then reduces halogenated compounds.

To understand this process in more detail and to gain insight into the functions of the different proteins and how the subunits function together, we started to purify this multiprotein complex. We use fast liquid protein chromatography with different columns in sequence. Fractions were tested for activity with a parallelized methyl viologen–based photometric activity assay and proteins are identified with highest sensitivity by mass spectrometry from active fractions.

Activity of RdhA was preserved after anion exchange and size exclusion chromatography. The fractions were also separated by Blue Native Polyacrylamide Gel electrophoresis (BN-PAGE) and non-natively by SDS-PAGE. In the BN-PAGE an intense band between 240 kDa and 480 kDa was found. The hypothesized size of the respiratory reductive complex is 341 kDa. SDS-PAGE showed intense bands around 50 kDa correlating with the size of RdhA monomer. Mass spectrometric analysis of the proteins in the band from BN-PAGE confirmed the presence of most of the complex proteins and in bands from SDS-PAGE the presence of RdhA was verified. Especially difficult to identify are the integral membrane proteins of the complex due to resistance of transmembrane peptides to fragmentation. We therefore developed and implemented a new strategy to identify transmembrane domains based on MS1 scans, with which we are currently trying to identify all complex proteins.

The transfer of electrons in a protein complex where electron donor and acceptor are in close proximity seems to be a fast mode in electron transport chains. Refinement of knowledge about this novel electron transport chain will be useful for applications in degradation of halogenated pollutants or biosensors.

References

[1] Kublik, A.; Deobald, D.; Hartwig, S.; Schiffmann, C.L.; Andrades, A.; von Bergen, M.; Sawers, R.G.; Adrian, L. (2016) Identification of a multiprotein reductive dehalogenase complex in *Dehalococcoides mccartyi* strain CBDB1 suggests a protein-dependent respiratory electron transport chain obviating quinone involvement, Environ. Microbiol. 18, 3044-56

Presentation: Wednesday, 8 March 2017 from 14:00 - 14:15 in room 12.

276/SMV

Morphogenic biomolecules: Why the marine polyp *Hydractinia echinata* cant live without his microbes

M. Rischer*[1], H. Guo[1], M. Roth[1], C. Weigel[1], C. Beemelmanns[1]
[1]*Hans Knöll Institut , Jena, Germany*

Introduction: Inter-kingdom interactions are often mediated by small molecules. They can act as virulence factors, have defensive character to protect against pathogens, or act as morphogenic stimulants. We have chosen the marine polyp *Hydractinia echinata* as a model system to study the chemical communication between the eukaryotic host and its associated microbiome [1]. The life cycle of the hydroid polyp *H. echinata* includes the bacterially induced transition of the motile larvae to the sessile reproductive phase (polyp). The larva develops into a primary polyp only in response to a chemical cue/specific molecule provided by associated environmental bacteria.

Objectives: We aim to analyze and characterize the respective microbial secondary metabolites and interspecies communication signals.

Materials and Methods: We used a culture-dependent and independent approach to identify associated microbes. We then prioritized the culture extracts using antimicrobial activity and larvae settlement assays. In addition, we sequenced the genomes of selected bacterial isolates to investigate the biosynthetic potential [2a;b]. We are currently aiming for characterization of the respective encoded natural products and settlement cues.

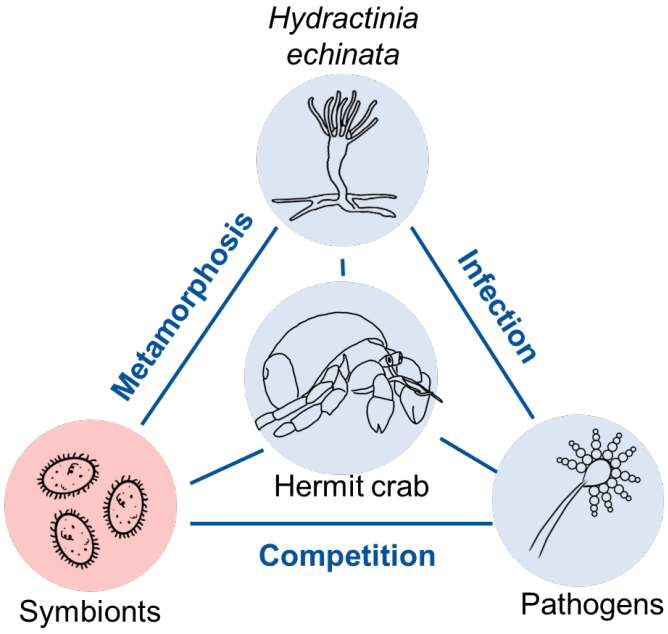
Results: Sequenced strains were analyzed using AntiSMASH revealing in particular NRPS biosynthesis gene clusters. Activity-guided analysis of antimicrobial strains allowed us to identify N-acylamino acids as the active components. By using a specific metamorphosis-assay, we prioritized our isolates and are currently characterizing the microbial signal responsible for the morphogenic activity.

Conclusion: *H. echinata* associated microbes induce larval settlement and metamorphosis. Preliminary data showed that the bacterial signal is part of the microbial biofilm and stable to a broad range of physical and enzymatic treatment. In addition, the inducing strains showed high antimicrobial activity against a broad range of microbial strains indicating a defensive function in this ecological system. The discovery of novel antimicrobial compounds highlights the chemical potential of this unexplored niche.

References

[1] Müller WA, Leitz T (2002) *Can. J. Zoo*, *80*, 1755–1771.
[2] a) Rischer M, Klassen J, Wolf T, Guo H, Shelest E, Clardy J, Beemelmanns C (2016) *Genome Announc* 4(1), e00003-16. b) Klassen JL, Wolf T, Rischer M, Guo H, Shelest E, Clardy J, Beemelmanns C (2015) *Genome Announc* 3(6), e01477-15.

Figure 1



Presentation: Wednesday, 8 March 2017 from 14:15 - 14:30 in room 12.

277/SMV

The secreted metabolome of *Streptomyces chartreusis* displays higher than expected diversity and strong nutrient-dependent regulation

C. Senges[1], A. Al-Dilaimi[2], D. Wibberg[2], A. Winkler[2], S. Schäkermann[1], M. Nowrousian[3], J. Kalinowski[2], J. Bandow*[1]
[1]*Ruhr-Universität Bochum, Applied Microbiology, Bochum, Germany*
[2]*Bielefeld University, Center for Biotechnology, Bielefeld, Germany*
[3]*Ruhr-Universität Bochum, Department of General and Molecular Botany,, Bochum, Germany*

Actinomycetes are masters of secondary metabolite production. Despite their fame their biosynthetic potential might still be underestimated. Combining genomics with untargeted data-dependent tandem mass spectrometry and molecular networking, we characterized the secreted metabolome of tunicamycin producer *Streptomyces chartreusis* NRRL 3882. The genome harbors 128 biosynthetic gene clusters predicted by antiSMASH. Revealing an unanticipated diversity of microbial chemistry, we detected more than 1000 distinct metabolites in culture supernatants, only ten of which were identified searching public databases. Approximately 40 additional identifications were based on literature, analytical standards, and manual annotation of mass and fragment spectra. Structures and functions of the vast majority of metabolites are unknown. The medium composition strongly affects the secreted metabolome. A number of secondary metabolites are produced iron-dependently, among them nine known and eight novel desferrioxamine siderophores aiding in iron acquisition. The structure and function of the other metabolites produced specifically under iron limitation are unknown. They might aid in iron acquisition or in adaptation to iron-limited conditions. Compared to chemically defined salt-based medium, in rich medium structural diversity was greater, total metabolite abundance was higher, and the average molecular weight was almost twice as high. Tunicamycins were only produced in rich medium, supporting a role of these antibiotics in nutrient defense. Metabolomic adaptation to environmental and cultivation conditions is more complex than previously expected. The findings of this study and, more broadly, the sensitivity of the established workflow enable further investigations into the ecological and physiological function of bacterial secondary metabolites.

Presentation: Wednesday, 8 March 2017 from 14:30 - 14:45 in room 12.

278/SMV

Skin-borne bacterial volatiles and their effect in bacterial interactions

M. C. Lemfack*[1], S. Rao Ravella[2], M. Kai[1], S. Schulz[2], B. Piechulla[1]
[1]*University of Rostock, Biochemistry, Rostock, Germany*
[2]*Technical University of Braunschweig, Organic Chemistry, Braunschweig, Germany*

The skin is the most exposed organ and serves as a barrier between the body and the external environment. Consequently, our skin is colonized by diverse species of microbes known as the skin microbiota (1). This microbiota inhibits pathogens, e.g. it has been shown that skin bacteria are able to produce secondary metabolites called bacteriocins, with antimicrobial properties against skin pathogens like Staphylococcus aureus (2). Skin bacteria also produce a large variety of small metabolites: due their high vapour pressure they are volatile organic compounds (mVOCs) (3). Interestingly, the potential effects of these mVOCs on the skin microbiota have largely been overlooked. Therefore, we performed the first comprehensive analysis of skin-borne bacterial volatiles using GC-MS and tested the effect of these volatiles on bacterial species of the skin community. We found that the typical skin-resident bacteria are able to produce a broad range of chemical classes of VOCs. While some of these compounds were specific to certain strains, some were strain/species unspecific. Among the specific volatiles, two novel natural compounds, schleiferons A and B, were isolated from Staphylococcus schleiferi isolates. When tested, these compounds inhibited differentially a broad range of Gram-positive bacteria with the minimal inhibitory concentrations ranging from 35 to 560 µM for schleiferon A and 141 to 3380 µM for schleiferon B. Schleiferons A and B also affected quorum sensing of Gram-negative bacteria. Especially, they inhibited prodigiosin production and bioluminescence emission of Serratia marcescens and Vibrio harveyi respectively. Schleiferons thus, turned out to be good candidates for further investigations for antibiotic and anti-virulence therapies.

References

[1] Grice and Segre. Nat. Rev. Microbiol. 2011
[2] Götz et al. Int. J. Med. Microbiol. 2014
[3] Verhulst et al. Malar. J. 2009

Presentation: Wednesday, 8 March 2017 from 14:45 - 15:00 in room 12.

WORKSHOP 46
Pathomechanisms of Shigatoxin-producing Escherichia coli (FG ZO / DVG)
08 March 2017 • 13:30 – 15:00

279/ZOV

***Escherichia coli* O157:H7 strain EDL933 expresses several *O*-acetyl esterases being involved in sialic acid catabolism**

N. Saile*[1], A. Voigt[1], S. Nübling[1], S. Kessler[1], T. Stressler[2], J. Klumpp[3], L. Fischer[2], H. Schmidt[1]
[1]*University of Hohenheim, Food Microbiology and Hygiene, Stuttgart, Germany*
[2]*University of Hohenheim, Biotechnology and Enzyme Science, Stuttgart, Germany*
[3]*ETH Zurich, Food, Nutrition and Health, Zurich, Switzerland*

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 are foodborne intestinal human pathogens which are responsible for severe diseases such as the hemolytic uremic syndrome. The main virulence factor is the ability to produce one or more Shiga Toxins (Stx). The corresponding toxin genes are located in the genome of lambdoid prophages. EHEC O157:H7 strain EDL933 harbours seven prophage-located genes, which we designated *nanS*-p, whose functions were previously unknown. The nomenclature is based on the similarity of these genes to chromosomal *nanS*, encoding a 5-*N*-acetyl-9-*O*-acetyl neuraminic acid (Neu5,9Ac$_2$) esterase, and their localisation on prophages (p). Neu5,9Ac$_2$ is a sialic acid derivative which is found in mucus externally bound to the glycoprotein mucin. Degradation of Neu5,9Ac$_2$ by NanS to 5-*N*-acetyl neuraminic acid (Neu5Ac) is the initial step of energy supply from Neu5,9Ac$_2$.
Objectives: The aim of our study was to characterize several NanS-p proteins from EHEC O157:H7 strain EDL933 and to investigate their function.
Materials and methods: We first sequenced the genome of our laboratory O157:H7 EDL933 strain and deleted *nanS* and sequentially all 7 *nanS*-p (*nanS*-p1-*nanS*-p7). Furthermore we deleted *nanS* in an apathogenic *E. coli* C600 strain, not carrying any *nanS*-p allele. All strains were grown on Neu5,9Ac$_2$ as sole carbon source. NanS-p1, NanS-p2 and NanS-p4 were recombinantly expressed and the enzymatic characteristics were determined. Moreover, the growth media for deletion mutants EDL933Δ*nanS*Δ*nanS*-p1-p7 and C600Δ*nanS* were complemented with the recombinant proteins.
Results: As expected, after deletion of *nanS* in *E. coli* C600 the mutant strain C600Δ*nanS* lost its ability to grow on Neu5,9Ac$_2$,

quite contrary to EDL933Δ*nanS* that grew similar as the wildtype strain EDL933. By deletion of 6 *nanS*-p alleles, the strain lost its ability to grow with Neu5,9Ac$_2$ as a carbon source. Moreover NanS-p1, NanS-p2 and NanS-p4 were enzymatically active with Neu5,9Ac$_2$ and in mucin from bovine submaxillary gland. The properties of the investigated enzymes are only slightly different but all of them could complement EDL933Δ*nanS*Δ*nanS*-p1-p7 and C600Δ*nanS* to regain the ability to grow with Neu5,9Ac$_2$ when added extracellularly.
Conclusion: We hypothesize that the redundant enzymes may be a prerequisite for good growth in the large intestine by facilitating the use of mucus-derived sialic acids as carbon sources.

Presentation: Wednesday, 8 March 2017 from 13:30 - 13:45 in room 7-8.

280/ZOV
Making Shiga toxin-producing *Escherichia coli* stick: Adhesive factors in *eae*-negative serogroup O91
S. Kahnert*[1], S. Bletz[1], S. Kampmeier[1], B. Middendorf[1], H. Karch[1], A. Mellmann[1]
[1]*Institute of Hygiene, University Hospital Muenster, Münster, Germany*

Introduction: Shiga toxigenic *Escherichia coli* (STEC) are potentially lethal enteric pathogens. Adhesion to human intestinal epithelial cells is crucial for pathogenicity and in most isolates facilitated by the *eae* gene encoding intimin. However, isolates lacking *eae* can also cause severe diseases including the hemolytic-uremic syndrome (HUS). Out of these *eae*-negative serogroups, O91 is the most common in human isolates, and serotype O91:H21 is frequently associated with HUS.
Objectives: To elucidate the presence and diversity of non-intimin adhesive genes in O91, we analyzed genome sequences from a large collection of O91 isolates. Moreover, we investigated possible associations of adhesive genotypes with serotypes and disease severity.
Materials & Methods: The collection of 118 epidemiologically unrelated O91 strains comprised isolates from patients with HUS (n=8), watery (n=84) or bloody diarrhea (n=7) or asymptomatic carriage (n=10) from 8 countries (clinical course not known for 9 samples). Isolates covered 4 serotypes (O91:H14/H$^-$ [n=88], O91:H21 [27], O91:H10 [2], O91:H8 [1]) and 11 multilocus sequence types (ST). A list of 87 known adhesion-related genes was compiled from literature on STEC pathogenicity; reference sequences of the genes were downloaded from the NCBI website. We queried these targets in both *de novo*-assembled sequences and reference-guided mappings of raw reads against the respective genes using the SeqSphere$^+$ software (Ridom GmbH, Muenster, Germany). Genes with ≥90% nucleotide similarity and ≥30% overlap to the reference sequence were rated as present; allelic variations (≥1 SNP difference) were also noted.
Results: All isolates carried between 46 and 55 genes related to adhesion, with a total of 61 different genes being found. Fimbrial clusters *csg, ecp, fim, flh, fli*, and *hcp* were detected in all strains; non-fimbrial genes *cadA, hns, iha*, and *ompA* were also common (in 115 to 118 samples). The two most prevalent serotypes (O91:H14 and O91:H21) shared 52 adhesive genes; in 44, however, the alleles differed and showed a serotype-specific distribution. Other adhesion-related genes were also associated with specific serotypes: *eibG* was exclusive to O91:H14 and *saa* was nearly exclusively present in O91:H21; hence it was also significantly associated with HUS (p=0.002). All 12 ST33 isolates positive for *eaeH* also harbored *aidA-I* and a specific *ehaA* allele, features not found in any other strains.
Conclusion: Non-intimin adhesive genes are common in O91. The majority of these genes exhibit different alleles, frequently serotype-specific, underlining that O91 is not a homogenous group of isolates. Significant associations of genotypes and disease severity might enable risk assessment in the future.

Presentation: Wednesday, 8 March 2017 from 13:45 - 14:00 in room 7-8.

281/ZOV
Characterization of a novel *stx*$_{2f}$-encoding prophage in enterohemorrhagic *Escherichia coli* of serotype O26:H11
B. Middendorf*[1], S. Kampmeier[1], B. Bunk[2], C. Spröer[2], M. Bielaszewska[1], H. Karch[1], A. Mellmann[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*
[2]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Microbial Ecology and Diversity Research, Braunschweig, Germany*

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC), a highly pathogenic subgroup of intestinal pathogenic *E. coli*, can cause hemolytic uremic syndrome (HUS) as severe post-infective complication. The major EHEC virulence factor Shiga toxin (Stx) comprises two antigenic forms which can be further classified into subtypes (Stx1a/c/d and Stx2a-h, respectively). Epidemiologic data indicate that strains producing Stx2a, Stx2c, or Stx2d are more often associated with severe disease than strains producing Stx1, Stx2b, or any of the remaining Stx2-specific subtypes. However, recent reports have shown that human infections with rather mild symptoms caused by *stx*$_{2f}$-encoding *E. coli* strains are becoming more frequent. Besides Stx, EHEC can encode additional toxins such as the inhibitory cyclomodulin cytolethal distending toxin (Cdt). Both virulence factors are typically encoded on lambdoid prophages embedded in the bacterial core genome.
Objectives, Materials & Methods: An *stx*$_{2f}^+$/*cdt*$^+$ EHEC of serotype O26:H11 was isolated from stool of a 7-month old female patient suffering from HUS. Further enteric bacterial or viral pathogens were not identified. A follow-up isolate of the same serotype but lacking both toxins could be obtained from this patient. In addition, *stx*$^-$/*cdt*$^-$ strains of serotype O26:H11 were isolated from the stool of four out of six family members with recurrent episodes of diarrhea. All isolates were subjected to core genome multilocus sequence typing (cgMLST) that was performed after genome sequencing using the MiSeq®-System (Illumina) and subsequent data analysis with the SeqSphere+ software (Ridom GmbH). Furthermore, *stx*$_{2f}^+$/*cdt*$^+$ and *stx*$_{2f}^-$/*cdt*$^-$ isolates of the index patient were subjected to SMRT sequencing (Pacific Biosciences) to generate full genome sequences for a detailed analysis of toxin-encoding prophage regions.
Results: According to cgMLST data all identified isolates of serotype O26:H11 were closely related indicating an intra-familial transmission of the strain. Within the genome of the initial EHEC isolate all classical integration sites for *stx*-phages (*wrbA*, *yehV*, *yecE*, *sbcB*, and z2755) were unoccupied. However, a so far undescribed ~68 kb prophage region with a GC-content of 48.68 % and 68 open reading frames including *stxA*$_{2f}$, *stxB*$_{2f}$, *cdtA*, *cdtB*, and *cdtC* was identified in proximity to the tmRNA gene *ssrA*. According to preliminary data, this region is partially excised in the follow-up isolate of the index patient leading to a loss of genes encoding both toxins.
Conclusion: Contrary to previous data, *stx*$_{2f}$-prophages can be associated with EHEC of major serotypes such as O26:H11 and these strains are able to cause HUS in susceptible patients. In this context, the genetic linkage of two major toxins might contribute to the pathogenic potential of the corresponding prophage and indicates a new emerging pathogen variant.

Presentation: Wednesday, 8 March 2017 from 14:00 - 14:15 in room 7-8.

282/ZOV

***In vitro* interaction of enterohemorrhagic *Escherichia coli* outer membrane vesicles with the intestinal microbial flora**

C. Correa-Martinez*[1], K. A. Jarosch[1], M. Bielaszewska[1], H. Karch[1], A. Mellmann[1], B. Middendorf[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*

Introduction: Outer membrane vesicles (OMVs) are nanostructures released by gram-negative and gram-positive microorganisms containing a wide range of cargo whose relevance in infection is not yet fully understood. The effects of pathogenic bacteria on the host's intestinal cells and the subsequent development of an immune response have been largely studied. However, interactions between infecting and commensal bacteria might also play a decisive role in the successful establishment of an infection, as the disruption of the gut microbiota undermines the intestinal barrier immunity. Furthermore, infecting bacteria seem to achieve a pathogenic effect not only by directly interacting with target cells but also by producing toxin-loaded OMVs capable of interacting with other prokaryotes and serving as decoy targets for antibodies and phages.
Objectives: The aim of this ongoing study is to investigate the influence of OMVs produced by strains of enterohemorrhagic *Escherichia coli* (EHEC) on the growth of representative strains of the intestinal microbiota as well as to further characterize the specific components responsible for the observed effects.
Materials and Methods: OMVs were isolated from cultures of *stx*-negative derivatives of EHEC strains with serotypes O104:H4 and O26:H11 in the late stationary phase, as described in previous studies that report a maximal OMV production by *Shigella dysenteriae* at this time point. The amount of vesicles present in the final preparations was indirectly measured by determining the total protein concentration with a modified Bradford's assay. Commensal reference strains (*Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus, Proteus mirabilis, Enterobacter cloacae, Klebsiella pneumoniae* and *E. coli*) were subsequently co-cultivated for 10 hours with OMVs at equivalent concentrations and analyzed for CFU/ml and OD_{600} hourly in order to assess their growth.
Results: In preliminary experiments, *E. coli* C600 showed an initial CFU/ml increase during the first two hours of co-incubation with OMVs of O104:H4, proceeding to drop continuously for the next 8 hours. OD_{600} also decreased progressively in the course of the co-incubation, whereas the control cultures displayed a normal growth rate. This decrease of the CFU/ml and OD_{600} values below initial levels indicates a lytic effect of the OMVs on the target cells which will be further tested on the different reference strains.
Conclusions: According to our preliminary results, OMVs of pathogenic *E. coli* strains have an inhibitory, possibly bactericidal effect on several bacteria of the physiological intestinal flora. This would represent a novel way of indirect bacterial interaction that facilitates the establishment and progress of infections.

Presentation: Wednesday, 8 March 2017 from 14:15 - 14:30 in room 7-8.

283/ZOV

Trafficking and cell injury by EHEC O157 virulence factors associated with outer membrane vesicles

A. Bauwens*[1], C. Rüter[2], L. Greune[2], M. A. Schmidt[2], A. Mellmann[1], H. Karch[1], M. Bielaszewska[1]
[1]*Universität Münster, Institut für Hygiene, Münster, Germany*
[2]*Universität Münster, Center for Molecular Biology of Inflammation, Münster, Germany*

Introduction: Enterohemorrhagic *Escherichia coli* (EHEC) O157, the most common cause of the life-threatening hemolytic uremic syndrome in children, secrete several virulence factors which may contribute to the pathogenesis of this disease via microvascular endothelial injury.
Outer membrane vesicles (OMVs) are involved in the virulence of a variety of bacterial pathogens but their role in the pathogenesis of infections caused by EHEC O157 is poorly understood.
Objectives: In this study, we investigated OMVs secreted by EHEC O157 for the presence of virulence factors, interactions with cells involved in the pathogenesis of human infections, and mechanisms of cell injury.
Materials and Methods: We analyzed OMVs from two EHEC O157 clinical isolates (O157:H7 and sorbitol-fermenting O157:H-) for their uptake, trafficking, and mechanism of injury to microvascular endothelial cells (human brain microvascular endothelial cells, human renal glomerular endothelial cells) and intestinal epithelial cells (Caco-2). To achieve our goals we used a fluorometric assay, electron and confocal laser scanning microscopy, immunoblotting, and bioassays.
Results: We demonstrate that O157 OMVs contain a cocktail of major toxins of EHEC O157 as well as flagellin. The toxins are internalized by cells via endocytosis of OMVs. Following the cellular uptake, the toxins separate from OMVs and are trafficked via different pathways to their target compartments including the cytosol, nucleus, and mitochondria. The OMV-delivered virulence factors cause distinct kinds of injury to the target cells that ultimately leads to cell death.
Conclusions: Our findings of EHEC O157 OMVs as carriers and delivery tools of biologically active virulence factors into cells affected during EHEC infections provide new insights into the pathogenesis of EHEC O157-mediated human diseases.

Presentation: Wednesday, 8 March 2017 from 14:30 - 14:45 in room 7-8.

284/ZOV

Phenotypic comparison of enterohemorrhagic *Escherichia coli* O104:H4 with enteroaggregative *Escherichia coli*

N. Haarmann*[1], P. Berger[1], A. Bauwens[1], M. Berger[1], H. Karch[1], A. Mellmann[1]
[1]*University Hospital Münster, Institute of Hygiene, Münster, Germany*

Introduction: *Escherichia coli* O104:H4 was identified as the pathogen causing the largest outbreak of bloody diarrhea and hemolytic uremic syndrome (HUS) in Germany in 2011. It is hypothesized that the exceptional pathogenicity of this strain derives from the hybrid character of this strain i.e. from the combination of virulence determinants originating from enterohemorrhagic *E. coli* (EHEC; Shiga toxin) and enteroaggregative *E. coli* (EAEC; aggregative adherence fimbriae I and Dispersin), respectively. Here, we phenotypically compared the outbreak strain with other typical EAEC strains in order to assess the contribution of enteroaggregative virulence factors on its pathogenicity.
Method: In this study, we compare *E. coli* O104:H4 C227-11 Φcu (cured of the Shiga toxin-encoding phage) with the prototypical EAEC strains 55989 and 042. Growth characteristics were monitored in LB medium, in a cell culture medium (DMEM) and in a medium mimicking the conditions in the colon (SCEM). Moreover, the production of different virulence determinants were

measured using semi-quantitative Western blot analysis. Finally, the strains were further phenotypically characterized using qualitative and quantitative adherence, biofilm, and cell cytotoxicity assays.
Results: The growth experiments showed similar growth characteristics for the strains C227-11 Φcu and 55989. Even though EAEC 042 showed the lowest final bacterial density under the conditions tested, it produced twice as much Dispersin as EAEC 55989, and three fold more than C227-11 Φcu. The qualitative adherence assay confirmed the typical stack-bricked pattern of EAEC. The biofilm formation of EAEC 042 in DMEM was five (55989) to twelve (C227-11 Φcu) times higher than in the other strains but two (55989) to four (C227-11 Φcu) times lower in SCEM.
Discussion: Our phenotypical characterization correlates well with the close genotypic relationship of C227-11 Φcu and EAEC 55989. Further detailed analyses of cytotoxicity and quantitative adherence assay will reveal more information about the influence of enteroaggregative determinants of the outbreak strain.

Presentation: Wednesday, 8 March 2017 from 14:45 - 15:00 in room 7-8.

WORKSHOP 47
Microbial pathogenesis of enteric infections (FG GI / FG MP)
08 March 2017 • 13:30 – 15:00

285/GIV
Analysis of host microRNA function uncovers a role for miR-29b-2-5p in *Shigella* capture by filopodia
U. Sunkavalli*[1], R. J. Silva[2], C. Aguilar[1], M. Sharan[1], A. R. Cruz[2], C. Tawk[1], C. Maudet[1,3], M. Mano[2], A. Eulalio[1]
[1]*Institute for Molecular Infection Biology, Host RNA Metabolism, Wuerzburg, Germany*
[2]*UC-BIOTECH, University of Coimbra, Center for Neuroscience and Cell Biology, Coimbra, Portugal*
[3]*Institut Pasteur, Biology of Infection Unit, Paris, France*

Introduction: MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression by repressing the expression of cellular mRNAs exhibiting partially complementary sequences. In addition to their pervasive and well-established functions in physiological and pathological processes, recent evidence uncovered the role of miRNAs during bacterial infection. However, the role of miRNAs in the interplay between the bacterial pathogen *Shigella flexneri* and the host cell remains unexplored. To address this, we performed a high-throughput functional screening using a library of human microRNA mimics to identify miRNAs that effect *Shigella* infection. *Shigella,* a gram-negative bacterium from the Enterobacteriaceae family, is a common causative agent of food-borne diseases.
Objectives: We identified miR-29b-2-5p among the miRNAs that increase *Shigella* infection. Our goal was to characterize the mechanism(s) by which this miRNA is modulating the interaction between the host cell and *Shigella*.
Results: We characterized the role of miR-29b-2-5p on *Shigella* infection and showed its regulatory role both in increasing bacterial binding to host cells and intracellular replication. Using a combinatorial approach of transcriptomic analysis coupled with targeted siRNA screening, we identified UNC5C as a target of miR-29b-2-5p with a role in *Shigella* infection. MiR-29b-2-5p, through the repression of UNC5C, strongly enhances filopodia formation via a mechanism dependent on the RhoGTPases RhoF and Cdc42. We show that the increase of filopodia favors bacterial capture and consequent *Shigella* invasion of host cells. Interestingly, the levels of miR-29b-2-5p, but not of other mature microRNAs from the same precursor, are decreased upon *Shigella* replication at late times post-infection, through degradation of the mature microRNA by the exonuclease PNPT1.
Conclusion: Our results indicate that the relatively high basal levels of miR-29b-2-5p at the start of infection ensure efficient *Shigella* capture by host cell filopodia. The decrease of the miR-29b-2-5p levels later during *Shigella* replication may constitute a bacterial strategy to favor a balanced intracellular replication to avoid premature cell death and favor dissemination to neighboring cells. Overall, this study highlights the unappreciated role of miRNAs, in particular of miR-29b-2-5p, in the interaction of *Shigella* with host cells.

Presentation: Wednesday, 8 March 2017 from 13:30 - 13:45 in room 6.

286/GIV
Genome and methylome variability of the *cag* pathogenicity island-carrying *Helicobacter pylori* challenge strain BCM-300 during early human infection
S. Nell[1], J. Krebes[1], I. Estibariz*[2,1], B. Bunk[3], G. Del Giudice[4], J. Overmann[3], Y. Song[5], C. Spröer[3], I. Yang[1], T. Wex[6], J. Korlach[5], P. Malfertheiner[6], S. Suerbaum[2,1]
[1]*Institute of Medical Microbiology and Hospital Epidemiology, Hannover, Germany*
[2]*Max von Pettenkofer Institut, Munich, Germany*
[3]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[4] *GSK Vaccines, Siena, Italy*
[5]*Pacific Biosciences, MenloPark, California, United States*
[6]*Otto-von-Guericke University, Magdeburg, Germany*

Helicobacter pylori undergoes rapid *in vivo* evolution but, so far, very little is known about genome and methylome variability during the initial phase of infection. Challenge studies in human volunteers offer the unique possibility to study the genetic adaptation of this uniquely versatile pathogen during the early phase of infection under highly standardized conditions. As part of a vaccine trial (VP992), twelve human volunteers were treated either with a prophylactic vaccine candidate containing three recombinant *H. pylori* proteins (CagA, VacA, and NAP) or placebo; and subsequently challenged with the *cag*PAI-positive *H. pylori* strain BCM-300. We used SMRT® sequencing technology to determine the genome sequences and methylomes of the *H. pylori* challenge strain BCM-300 and 12 reisolates obtained from gastric biopsies of the volunteers after 12 weeks or, in one case, 62 weeks post-infection.
Whole genome comparison revealed an average mutation rate of $5.2 \times 10{-}6$ mutations per site per year, which is in agreement with the majority of previous studies during chronic infection (Kennemann *et al*., 2011; Didelot *et al*., 2013; Furuta *et al*., 2015), but differs markedly from the results of one recent publication (Linz *et al*., 2014). Three reisolates of the vaccine group acquired premature stop codons within *vacA*, and therefore, lost the ability to induce vacuolization. The frequent inactivation of *vacA* in the vaccine group may be a result of immune-mediated selection induced by the vaccination. In addition, three reisolates lost the functionality of the *cag*PAI during infection.
Methylome analysis of BCM-300 and the reisolates identified a total of 15 methylated motifs. Eleven of these were assigned to known methyltransferase (MTase) activities by sequence homology. Three of the novel motifs could be assigned to previously uncharacterized MTases via inactivation of candidate genes. Inter-strain variability in the methylomes was observed, resulting from phase variation of two MTase genes. The length of two homopolymeric repeat regions within the sequence of one novel phase-variable MTase mediated a reversible ON/OFF switching of the enzyme activity, as well as a sequence specificity switch, similar to what was described in previous publications (Krebes *et al*., 2014). Phase variation of MTase genes might

confer a selective advantage in adaptation to different human hosts.
A key result of the study is the observation that the function of important *H. pylori* virulence factors including *cag*PAI and vaculating cytotoxin VacA was lost at a remarkable frequency during early human infection. This loss of virulence was previously observed in animal models, but not assumed to happen during human infection. The *H. pylori* methylome varies during infection and the implications of this variation will be discussed.

Presentation: Wednesday, 8 March 2017 from 13:45 - 14:00 in room 6.

287/GIV
Novel inhibitors for the New Delhi Metallo-beta-Lactamase 1
R. Nitiu[1], C. Daschkin[1], G. Popowicz[2], J. Herrmann[3], R. Müller[3], M. Sattler[2], M. Gerhard[1], H. Meyer*[1]
[1]*Technische Universität München, Institut für medizinische Mikrobiologie, Immunologie und Hygiene, München, Germany*
[2]*Helmholtz Zentrum München, Institute for Structural Biology, Neuherberg, Germany*
[3]*Helmholtz-Institut für Pharmazeutische Forschung Saarland, Saarbrücken, Germany*

Question: The New Delhi Metallo-beta-Lactamase 1 (NDM-1) confers antibiotic resistance to all beta-lactam antibiotics and was initially described in 2009. It has reached immediate attention due to its exceptionally broad substrate promiscuity on the one hand and its rapid dissemination both across the globe as well as between bacterial species, in particular *Enterobacteriacaea*. Moreover, being frequently plasmid encoded in combination with various resistances against most other classes of antibiotics, NDM-1 positive infections are extremely difficult to treat and pose an enormous threat to human health. Inhibition of the NDM-1 activity is expected to broaden the treatment options against NDM-1 positive pathogens and thereby meet this growing medical need.
Method: In our previous work, we have characterized the NDM-1 and found that both Zn2+ concentration and substrate type massively influence the efficacy of a reference inhibitor of the NDM-1. On this basis, we have set up a biochemical screening assay platform for NDM-1 inhibitors, which reflects the pathophysiology of infection and therapy. The included assays are validated against the parameters specified by the European Lead Factory and meet all criteria.
Results & Conclusion: Using this assay platform, we screened the library of natural compounds purified form Myxobacteria and identified two natural compound classes, which inhibit the NDM-1 activity with IC_{50} values in the single digit µM range. These natural compound inhibitors serve as tool compounds for a pharmacophore approach, which was complemented by a fragment based inhibitor screen. This endeavor resulted in the identification of an inhibitory scaffold which shows promising biochemical and biological activity against serine- and metallo-beta-lactamases.

Presentation: Wednesday, 8 March 2017 from 14:00 - 14:15 in room 6.

288/MPV
Engagement of CEACAM receptors by *Helicobacter pylori* modulates immune responses
A. Javaheri[1], R. Mejías Luque[1], T. Kruse[2], K. Moonens[3], I. Asche[4], N. Tegtmeyer[4], R. Haas[5], S. Backert[4], H. Remaut[3], B. B. Singer[6], M. Gerhard*[1]
[1]*Technische Universität München, Institut für Medizinische Mikrobiologie, Immunologie und Hygiene, Munich, Germany*
[2]*Imevax GmbH, Munich, Germany*
[3]*Structural Biology Research Center, VIB, Brussels, Belgium*
[4]*Friedrich Alexander University Erlangen, Erlangen, Germany*
[5]*Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Munich, Germany*
[6]*University Duisburg-Essen, Institute of Anatomy, Medical Faculty, Essen, Germany*

Helicobacter pylori (H. pylori) causes chronic active gastritis and is a major risk factor for the development of duodenal and gastric ulcers as well as gastric adenocarcinoma and lymphoma. Adhesion of the bacterium to the gastric epithelium is essential for the establishment of the infection. Specific adhesin-receptor interactions allow the pathogen to tightly bind to its target cells, thereby facilitating the colonization of the host tissue as well as the delivery of virulence factors. Here, the Type IV secretion system (T4SS) plays an essential role in gastric carcinogenesis by delivering the oncogenic protein CagA into gastric epithelial cells and thus activating inflammatory signaling pathways involved in the development of cancer. Interestingly, *H. pylori* T4SS can directly activate signaling cascades, such as canonical NF-κB, independently of CagA, suggesting other components of the T4SS to be important in the de-regulation of host signaling. We found that *H. pylori* has the capacity to bind to the CEACAM family of receptors, and identified HopQ as the adhesin of *H. pylori* that mediates CEACAM ligation. We further resolved the crystal structure of HopQ and the CEACAM binding domain. HopQ-mediated binding is necessary for CagA translocation, as CagA translocation can be abolished be interfering with the HopQ-CEACAM interaction through competition using HopQ derived peptides or CEACAM blocking antibodies. Further, we observed activation of alternative NF-κB in gastric epithelial cells *in vitro* and *in vivo* upon *H. pylori* infection, which was dependent on a functional T4SS, but independent on the presence of CagA. Notably, activation of alternative NF-κB highly influenced the inflammatory response to *H. pylori*, contributing to gastric pathology induced by the bacterium. Together, our results suggest that HopQ-mediated adherence as well as T4SS-dependent activation of alternative NF-κB play a major role in the pathogenesis of *H. pylori* infection. This novel interaction mode may also enable the development of future intervention strategies.

Presentation: Wednesday, 8 March 2017 from 14:15 - 14:30 in room 6.

289/MPV
Novel tools for the expression and characterization of adhesive structures in bacteria
N. Hansmeier[1], K. Miskiewicz[1], M. Hensel[1], T. Sterzenbach*[1]
[1]*Universität Osnabrück, Biologie/Chemie, Osnabrück, Germany*

Introduction: Adhesins play a crucial role for pathogenicity and dissemination of *Salmonella* and other pathogens. *Salmonella enterica* serovar Typhimurium alone encodes for 13 fimbrial, three autotransported and two T1SS-secreted adhesins. Since adhesins are generally tightly regulated to prevent their untimely expression, most of them are difficult to express under laboratory conditions.
Objectives: To overcome this burden, we developed novel tools for the expression, functional characterization and modification of adhesive structures.
Materials and Methods: Adhesins were expressed under the control of tetracycline or acetylsalicylate inducible promotor

elements and visualized by atomic force and transmission electron microscopy. Epitope tags were inserted into fimbrial subunits. Functionality was confirmed by adhesion assays.
Results: By using simple and versatile tools, we managed to express and visualize for the first time the adhesiome of *S.* Typhimurium. We could identify clearly distinguishable clusters of appearance. Furthermore, we identified permissive sites for epitope tagging of fimbriae. These insertions into several fimbrial subunits did not interfere with either their assembly or functionality. This simplifies immunological detection, single molecule studies and biophysical analyses of cryptic adhesins even in the absence of specific antibodies. Finally we will present data discussing variations in binding properties of autotransporters from different serovars of *Salmonella*.
Conclusion: We developed a valuable set of tools that allows expression, functional characterization and visualization of so far cryptic adhesins from *Salmonella*. We see first evidence that these tool boxes are transferable to other Gram-negative bacteria and may become an important asset for research on pathogenicity.

Presentation: Wednesday, 8 March 2017 from 14:30 - 14:45 in room 6.

290/MPV
Adhesion of *Salmonella* to pancreatic secretory granule membrane major glycoprotein GP2 depends on FimH sequence variation
R. Kolenda*[1], M. Burdukiewicz[2], J. Schiebel[1], I. Szabo[3], A. Orłowska[4], J. Nitschke[1], A. Böhm[1], P. Schierack[1]
[1]*Brandenburg University of Technology Cottbus-Senftenberg, Institute of Biotechnology, Senftenberg, Germany*
[2]*University of Wrocław, Department of Genomics, Wrocław, Poland*
[3]*Federal Institute for Risk Assessment (BfR), National Salmonella Reference Laboratory, Berlin, Germany*
[4]*Wrocław University of Environmental and Life Sciences, Department of Biochemistry, Pharmacology and Toxicology, Wrocław, Poland*

Introduction: GP2 is specifically expressed on the surface of M cells and takes part in uptake of type 1 fimbriae (T1F)-positive bacteria and transport to underlying mucosal immune tissues. This process continues with local and systemic dissemination of bacteria. T1F are one of the most common adhesive organelles in the family of Enterobacteriaceae and important adhesion factors in *Salmonella* intestinal pathogenicity. The FimH protein is located on top of the T1F shaft and directly interacts with receptors.
Objectives: Several studies have shown that serotype-associated FimH variants can differ significantly in receptor recognition and this can lead to change in course of infection. Therefore, our aim was to investigate the role of FimH sequence variation on binding to GP2 isoforms from various hosts.
Materials and methods: Human and porcine GP2 isoforms were expressed in SF9 cells. *fimH* gene sequences from 128 *Salmonella* isolates from five serotypes of human, cattle, swine and chicken origin were determined. Expression of FimH protein in these isolates was tested with a static anti-FimH antibody adhesion assay. An isogenic system with one *Salmonella* strain was generated. A *fimH* deletion mutant was created and ten plasmids containing *fimH* variants were transformed into this mutant. Static adhesion assay with four human and two porcine GP2 isoforms, HRP, RNase B and anti-FimH antibody was performed. HEp-2 and IPEC-J2 cells expressing human and porcine GP2 isoforms were generated with use of lentiviral expression system. Next, infection assay with these cell lines and *Salmonella* isogenic model was carried out.
Results: Comparison of *fimH* gene sequences from *Salmonella* isolates revealed 11 sequence variants with 17 variable sites. T1F expression was dependent on serotype and isolation source. Cluster analysis revealed, that T1F expression is higher in strains from non-host-restricted compared to host-associated or host-restricted serotypes ($p<0.001$). In our isogenic model, binding to GP2 isoforms and standard proteins was FimH-variant and mannose dependent, and glucose independent. The high biding phenotype was observed in case of four FimH variants, low in case of three variants and no binding in case of three variants. In cell line infection assay, GP2-cell line expression dependent binding was observed in case of three FimH variants with high binding phenotype. No host-specific binding of FimH adhesins to GP2 was observed in static adhesion assay and cell line infection assay.
Conclusion: T1F expression varies among serotypes. T1F-expressing *Salmonella* can adhere to GP2 in FimH-variant dependent manner. Adhesion of FimH-positive bacteria to GP2 might be an additional entry route for *Salmonella* invasion. Future in vitro and in vivo studies can help to elucidate the role of FimH-GP2 interaction.

Presentation: Wednesday, 8 March 2017 from 14:45 - 15:00 in room 6.

POSTERSESSION
Archaea (FG AR)

291/ARP
Influence of heavy Metal Ions on haloarchaeal Biofilms
S. Völkel*[1], S. Frölsl[1], F. Pfeifer[1]
[1]*TU Darmstadt, Darmstadt, Germany*

Introduction: Biofilms are microbial communities embedded in a hydrated matrix consisting of extracellular polymeric substances (EPS) and represent the predominant lifestyle of microorganisms in natural habitats. Besides several other advantages, biofilms offer protection against environmental stress factors like pH-shifts, UV-light, or toxic metal ions. While most previous studies focused on bacterial biofilms, little is known concerning metal resistance in archaeal biofilms so far[1].
Objectives: This study focuses on the biofilm formation of *Halobacterium salinarum* R1 as well as biofilm-mediated resistance and structural changes in mature biofilms in the presence of various metal ions, *i.e.* copper, nickel and zinc.
Materials & Methods: The *Hbt. salinarum* R1 cells were grown in complex medium in the presence or absence of different metal ions. The biofilm formation under these conditions was quantified using a fluorescence-based adhesion assay. To investigate the biofilm-mediated resistance against metal-ions, a quantitative PCR (qPCR) method was employed. The qPCR studies were verified by Live/Dead staining and fluorescence microscopy. Metal-specific effects on the biofilm structures were investigated by use of confocal laser scanning microscopy (CLSM).
Results: Growth in the presence of subinhibitory concentrations of different heavy metal ions showed distinct responses regarding adhesion and biofilm formation. While growth with nickel and zinc ions led to an increased biofilm formation with up to 50 % stronger adhesion signals, the presence of copper ions resulted in a strong inhibition of cell adhesion and biofilm formation compared to untreated cells. One-day treatment with lethal metal concentrations exhibited a biofilm-mediated resistance with up to 80 % increased survival rates of these biofilms. Microscopic investigations of the biofilm structures showed bulky biofilm forms up to 25 µm in height with enlarged EPS amounts. In comparison untreated cells formed thinner biofilms with an average height of 15 µm. Growth inhibiting concentrations of nickel resulted in a strong cell adhesion with multilayered cells and a duplication of the biofilm mass.
Conclusion: The effects of heavy metal ions on sessile cells of *Hbt. salinarum* R1 are metal-specific and can both inhibit and increase biofilm formation. In the presence of lethal metal concentrations, mature biofilms exhibit increased cell survival rates and show alterations of the biofilm architecture and EPS

composition, which might serve as a possible resistance mechanism of haloarchaeal biofilms.

References

[1] Fröls S, Dyall-Smith M, Pfeifer F (2012). Environ Microbiol 14(12):3159-74

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

292/ARP

Investigation of the Biofilm Development regarding the EPS Production of *gfp*-expressing *Halobacterium salinarum* R1

J. Born*[1], R. Wloch[1], F. Pfeifer[1]
[1]*TU Darmstadt, Mikrobiologie und Archaea, Darmstadt, Germany*

Introduction: The existence as sessile communities on solid surfaces (biofilms) provides several advantages for the microorganisms. Embedded within a matrix of extracellular polymeric substances (EPS), the cells are protected against environmental influences, obtain a better nutrient supply and are located at good surroundings for horizontal gene transfer. The composition of the EPS in biofilms of bacteria is variable and includes substances like carbohydrates, proteins, lipids, or extracellular DNA (eDNA) in variable amounts [1]. In contrast, the formation of archaeal biofilms as well as the composition of their EPS are not very well investigated. One method is to visualize biofilms and EPS by confocal laser scanning microscopy (CLSM) by tagging the cells with a fluorescent reporter gene (*gfp*) and staining different EPS compounds with specific fluorescent dyes.
Objective: The aim of this study was to investigate biofilm structures and the composition of EPS using a *gfp*-expressing *Hbt. salinarum* R1, a halophilic archaeon.
Methods: *Hbt. salinarum* R1 cells expressing the gene of a high salt tolerant GFP [2] were grown to different ages. Individual components in the biofilms were stained with different fluorescent dyes and the multicellular biofilm structures visualized by CLSM. Integral compounds of the EPS were isolated and qualitatively and quantitatively analysed.
Results: The biofilm matrix of *Hbt. salinarum* R1 contains saccharides, proteins and eDNA. The major fraction of the EPS is composed of proteins followed by saccharides and eDNA. CLSM analysis showed a primary attachment of cells followed by growth of bulky tower-like microcolonies (up to 60 µm in height). Extracellular substances, containing glycoconjugates with terminal α-D-mannosyl and α-D-glucosyl groups, as well as N-acetyl-D-glucosamine and sialic acids, were found with a defined arrangement inside the microcolonies. The presence of eDNA was detected already after one day of biofilm growth. Proteins were found in all sections of the microcolonies. An increase of the amount of EPS was observed throughout biofilm growth.
Conclusion: The determination of EPS-compounds of sessile *gfp*-expressing *Hbt. salinarum* R1 cells in combination with staining of single EPS components with specific fluorescent dyes (visualized by CLSM) are a good tool to investigate the development of halophilic biofilms.

References

[1] Fröls, S. (2013). *Biochemical Society transactions* 41 (1): 393–398.
[2] Reuter, Ch. J.; Maupin-Furlow, J. A. (2004). *AEM* 70 (12): 7530–7538

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

293/ARP

Trh7: a potential transcriptional regulator in *Haloferax volcanii*

B. Hammerbacher*[1], S. Dunin-Horkawicz[2], A. Marchfelder[1]
[1]*Universität Ulm, Molekulare Botanik, Biologie II, Ulm, Germany*
[2]*University of Warsaw, Structural Bioinformatics Laboratory, Centre of New Technologies, Warsaw, Poland*

Regulation of gene expression allows rapid adaptation to changing environmental conditions with transcription regulation being one of the most common methods to manage gene expression. The archaeal transcription machinery shares lots of properties with the corresponding eukaryotic one and is commonly summarized as a more simple version of the eukaryotic apparatus. Similarities are for example multiple RNA polymerase subunits and the use of basal transcription factors for initiation and elongation (1-3). In the halophilic archaeon *H. volcanii* only a few transcriptional regulators have been described (4-7). To learn more about transcription regulators, we analysed the potential transcriptional regulator Trh7 (HVO_2507) in *H. volcanii*, Trh7 belongs to the Lrp/AsnC family of transcription regulators and is encoded upstream of HVO_2506, HVO_2505 and *atsC*. Its gene is located next to the gene for the carbamoyl phosphate synthetase (*carA,* HVO_2508). We were able to generate a *trh7* deletion strain, which we analysed in detail for its phenotypes. No severe differences in growth could be detected between wild type and *trh7* deletion cells. Investigation of transcript levels for the HVO_2505-2506 operon as well as the neighbouring gene *carA* in the deletion strain revealed that Trh7 has a possible regulatory influence on HVO_2506 and HVO_2505. In addition, it seems to regulate the expression of *carA*. The *carA* gene codes for the small subunit of carbamoyl phosphate synthetase (CP) in *H. volcanii*. Deletion of the *trh7* gene results in higher expression of *carA*. This indicates a negative regulatory influence of Trh7 on *carA*. To verify that Trh7 regulates expression of *carA* we expressed Trh7 as recombinant protein and will perform EMSA experiments with the DNA region upstream of the *carA* ORF.
Interestingly, a conserved structure has been identified in the 5´ UTR of the *trh7* mRNA. We will employ EMSA to investigate whether the recombinant Trh7 can bind to this RNA sequence and thereby possibly autoregulate its own expression.

References

[1] Gehring et al., 2016 J Bacteriol. 198(14):1906-1917
[2] Gindner et al., 2014 Extremophiles 18: 926-936
[3] Blombach et al., 2016 J Mol Biol. 428(12): 2592-606
[4] Hattori et al., 2016 J Bacteriol. 198(7):1077-86
[5] Johnsen et al., 2015 Environ Microbiol. 17(5):1663-76
[6] Rawls et al., 2010 J Bacteriol. PMC2981212
[7] Qi et al., 2016 Extremophiles 20(1):27-36

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

294/ARP

L-Rhamnose degradation in the halophilic archaeon *Haloferax volcanii*

A. Reinhardt*[1], U. Johnsen[1], P. Schönheit[1]
[1]*Christian-Albrechts-Universität zu Kiel, Institut für Allgemeine Mikrobiologie, Kiel, Germany*

Haloferax volcanii is able to grow on various hexoses, e.g. D-glucose and D-fructose, and pentoses, e.g. D-xylose and L-arabinose. The degradation pathways of these sugars have been elucidated (1,2,3,4). Here we report that *H. volcanii* is also able to utilize L-rhamnose (6-deoxy-L-mannose) which is an abundant deoxy-hexose in nature. Genes and enzymes involved in rhamnose uptake and degradation were studied by (I) transcriptional analyses of candidate genes, (II) cloning, homologous overexpression, purification and characterization of enzymes and

(III) proof of functional involvement of enzymes by growth studies of deletion mutants of the coding genes. Together, the data indicate that in *H. volcanii* rhamnose is taken up by an ABC-transporter and is oxidatively degraded to pyruvate and lactate involving rhamnose dehydrogenase, rhamnonate dehydratase, 2-keto-3-deoxy-rhamnonate dehydrogenase and 2,4-diketo-3-deoxy-rhamnonate hydrolase. This is the first detailed analysis of rhamnose catabolism in the domain of archaea.

References

[1] Johnsen U et al. (2009) J Biol Chem 284; 27290-27303
[2] Pickl et al. (2012) J Bacteriol 194; 3088-3097
[3] Johnsen U et al. (2013) Extremophiles 17; 897-909
[4] Sutter JM et al. (2016) J Bacteriol 198; 2251-2262

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

295/ARP

O_2 sensitivity of [Fe]-hydrogenase in the presence of the substrates

G. Huang*[1], K. Ataka[2], S. Shima[1]
[1]*Max-Planck-Institut für terrestrische Mikrobiologie, Group of Seigo Shima, Marburg, Germany*
[2]*Freie Universität Berlin, Physics, Berlin, Germany*

[Fe]-hydrogenase is found in many hydrogenotrophic methanogenic archaea. It can reversibly activate H_2 and transfers a hydride ion to methenyl-tetrahydromethanopterin (methenyl-H_4MPT^+), which is an intermediate in the methanogenic metabolism from H_2 and CO_2. [Fe]-hydrogenase is inhibited by CO, cyanide [1], isocyanides [2, 3] and Fe^{2+} [4], and inactivated by Cu^{1+} and H_2O_2 [4]. However, unlike most of [NiFe]-hydrogenase and [FeFe]-hydrogenase, [Fe]-hydrogenase is insensitive against O_2 in the absence of the substrates. Here, we report that [Fe]-hydrogenase is inactivated in the presence of reduced substrates (methylene-H_4MPT or methenyl-H_4MPT^+ + H_2) and O_2, whereas this enzyme is stable even under 100% O_2 in the absence of the substrates. These results suggest that O_2 reacted with a reduced intermediate of the catalytic cycle. Infrared spectroscopic analysis of the intrinsic CO bands indicated the [Fe] center of this hydrogenase was decomposed by the O_2 treatment in the presence of the substrates. This gives us a possibility to probe what kind of intermediates generated during the catalytic reactions and to further study the catalytic mechanism of [Fe]-hydrogenase.

References

[1] Lyon, E. J., Shima, S., Buurman, G., Chowdhuri, S., Batschauer, A., Steinbach, K. & Thauer, R. K. (2004) UV-A/blue-light inactivation of the "metal-free" hydrogenase (Hmd) from methanogenic archaea, *Eur. J. Biochem.* 271, 195-204.
[2] Shima, S. & Ataka, K. (2011) Isocyanides inhibit [Fe]-hydrogenase with very high affinity, *FEBS Letters.* 585, 353-356.
[3] Tamura, H., Salomone-Stagni, M., Fujishiro, T., Warkentin, E., Meyer-Klaucke, W., Ermler, U. & Shima, S. (2013) Crystal structures of [Fe]-hydrogenase in complex with inhibitory isocyanides: implications for the H_2-activation site, *Angew. Chem. Int. Ed.* 52, 9656-9659.
[4] Hidese, R., Ataka, K., Bill, E. & Shima, S. (2015) Cu^I and H_2O_2 inactivate and Fe^{II} inhibits [Fe]-hydrogenase at very low concentrations, *Chembiochem.* 16, 1861-1865.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

296/ARP

The importance of 10 paralogs of Origin Recognition Complex (ORC1) proteins for chromosome copy number regulation and fitness

K. Ludt*[1], J. Soppa[1]
[1]*Goethe University Frankfurt, Frankfurt am Main, Germany*

Much less is known about the DNA replication machinery of Archaea, compared to the counterparts in Bacteria and Eukaryotes. Many archaea contain more than one origin of replication, e.g. three origins were found to operate in two species of *Sulfolobus*. Archaea encode Origin Recognition Complex (ORC1) proteins, which are homologous to the eukaryotic proteins ORC 1 and Cdc6. The archaeal ORC1 proteins initiate replication by recognizing and binding of the origins of replication, similar to the eukaryotic ORC proteins.
In total, the genome of the halophilic archaeon *Haloferax volcanii* H26 contains six replication origins, four on the main chromosome and one each on the small chromosomes pHV1 and pHV3. As usual, *orc1* genes are located close to each origin. However, *H. volcanii* contains 16 *orc* genes, and thus 10 *orc* genes are not located close to origins and their function and use are unknown.
To elucidate the importance of *Haloferax volcaniis orc1* genes, 12 examples were chosen based on their expression levels (RNA-seq data) and their co-localization with origins of replication. Single deletion mutants of all genes were generated using the so called Pop-In-Pop-Out method. Subsequently, growth capabilities were compared with that of the wild-type, and the copy numbers of the three chromosomes were quantified in all mutants using Real Time PCR.
The results showed that most of the *orc1* genes are non-essential. However, three orc1 genes could not be deleted and thus turned out to be essential. Two of these essential genes are co-localized with origins of replication oriC3 and ori-pHV3, while the third essential *orc1* gene is far from any origin on the main chromosome. Copy number determination revealed that all seven non-essential ORC1 proteins influence the number of at least one replicon. However, the pattern was complex, e.g. the deletion of some *orc* genes influenced all three replicons (e.g. HVO_*0001*), while in one case only the replicon encoding the gene was changed and in two cases the two replicons not encoding the gene were changed. Growth analysis revealed that deletion of the six *orc1* genes that are co-localized with origins of replication has nearly no effect on growth rate or yield. In contrast, two deletion mutants show a gain of function phenotype under all tested conditions (Δ*2042* & ΔA0257), and two additional deletion mutants showed a gain of function phenotype only when glucose was used as carbon source (Δ*2133* & Δ*A0001*). These results revealed that also the ORC1 paralogs that are encoded far from origins are important for copy number control and fitness. It seems that a complex synergistic network exists that affects the origins of replication on all replicons, and that the relative importance of singe ORC1 paralogs changes with environmental conditions.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

297/ARP

Cell division of the Euryarchaeon *Haloferax volcanii.*

P. Nußbaum*[1], S. V. Albers[1]
[1]*Albert-Ludwigs Universität, Institut für Biologie II - Mikrobiologie Molekulare Biologie der Archaeen, Freiburg, Germany*

Cell division is the most important process for a cell to generate progenies. While this process has been under investigation in eukaryotic and bacterial cells for many years, cell division in Archaea still remains to be elucidated. Interestingly, different cell division mechanisms are found within the domain of Archaea. It is

known that Crenarchaeota in most instances divide by homologs of the ESCRT (Endosomal Sorting Complex Required for Transport) system found in Eukarya. On the other hand, almost all members of the Euryarchaeota possess FtsZ homologs and therefore divide in a bacterial manner. In contrast to bacteria, additional genes of the FtsZ/tubulin superfamily are often found in euryarchaeal genomes. For example seven genes that encode proteins from the FtsZ/tubulin superfamily are found in the model organism *Haloferax volcanii*. However, recently it was shown that not all FtsZ homologs of *H. volcanii* are involved in cell division but in cell morphogenesis (Dugging et al. 2015). Yet another interesting fact is that five genes encoding MinD homologs were identified in the genome of *H. volcanii*, but neither genes for minC nor minE were identified. This raises on one hand the question whether all MinD homologs are involved in the localization of FtsZ to mid cell and on the other hand if there are other proteins replacing the function of MinC and MinE.
The object of this project is to identify the specific function of the five MinD homologs and to reveal putative interaction partners of them to get a deeper insight in the cell division of Archaea.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

298/ARP
Purification and analysis of accessory gas vesicle proteins from *Haloferax volcanii*
K. Winter*[1], F. Pfeifer[1]
[1]*TU Darmstadt, Microbiology and Archaea, Darmstadt, Germany*

Introduction: Gas vesicles are proteinaceous and gas-filled nanostructures. Fourteen *gvp* genes are involved in gas vesicle formation. The two major structural proteins GvpA and GvpC are forming the ribbed protein wall, whereas the eight proteins GvpF through GvpM are accessory gas vesicle proteins. Except GvpH and GvpI, all of them are required for gas vesicle formation. Not very much is known about their function. They occur in the exponential growth phase prior to the presence of large amounts of GvpA. Heterologous production in *E. coli* is possible but can pose problems since halophilic proteins typically misfold and aggregate in conditions of low ionic strength. Purification of halophilic proteins from *E. coli is based o*n the recovery of misfolded proteins, followed by denaturation and refolding in hypersaline solutions. However, for gas vesicle proteins (Gvps) there is no proof of effective refolding by reason of lacking enzymatic activity. In the past, purification of Gvps from haloarchaea using a polyhistidine-tag was challenging because of low protein yields and impurity.
Objectives: We searched for suitable affinity tags to purify accessory gas vesicle proteins under native conditions in *Haloferax volcanii*.
Material & Methods: For purification, we choose the cellulose-binding-domain (CBD) from the CipB protein of *Clostridium thermocellum* [1] which was fused to the N-terminus of the gas vesicle proteins GvpL (L-CBD) and GvpM (M-CBD). Purification was performed with spin columns packed with microcrystalline cellulose and proteins were eluted by addition of ethylenglycol. Purity was proven by SDS-PAGE, coomassie staining and Western analysis.
Results: By using the cellulose binding domain it was possible to purify the two accessory gas vesicle proteins GvpL and GvpM with high purity and a proper protein yield. Purification of L-CBD revealed 6- to 10-fold higher protein yields in contrast to M-CBD, which is presumably due to the hydrophobic and aggregatory property of GvpM. Compared to the isolation via his-tagged proteins, both purification methods revealed similar amounts of total protein. However, the elution fractions of GvpLHis and GvpMHis contained many contaminants like the intrinsic protein PitA whereas L-CBD and M-CBD contained exclusively the desired proteins. The purified proteins were used to investigate protein-protein interactions that might occur during gas vesicle assembly.
Conclusion: Purification of various accessory gas vesicle proteins under native conditions opens the possibility to study their structure and function during the gas vesicle assembly.

References
[1] Irihimovitch V, Ring G, Elkayam T, Konrad Z, Eichler J (2003). Extremophiles 7(1):71-7.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

299/ARP
Development of a genetic reporter system to study selenium-dependent gene regulation and selenocysteine biosynthesis in *Archaea*
V. Quitzke*[1], M. Rother[1]
[1]*Technical University Dresden, Institute of Microbiology, Dresden, Germany*

Proteins containing selenocysteine (sec) occur in all three domains of life, *Archaea*, *Bacteria*, and *Eukarya*. The archaeal model organism *Methanococcus maripaludis* contains several selenoproteins involved in its primary metabolism, hydrogenotrophic methanogenesis. Interestingly, the organism also contains cysteine- (cys-) containing isoforms of all selenoproteins (with the exception of formate dehydrogenase), which are formed when selenium is scarce or when the path of sec synthesis is disrupted. While formate-dependent growth is impaired under such conditions, the organism can still grow on H2+CO2. How selenium availability is sensed and the information transduced to effect antagonistic regulation of the selenoprotein genes and their cys-encoding isogenes, is poorly understood.
In order to study selenium-dependent gene regulation in *M. maripaludis*, a transcriptional fusion comprising the promoter of *frcA* (encoding a subunit of the selenium-free, F420-reducing, hydrogenase Frc), *PfrcA*, and *bla* (encoding b-lactamase from *E. coli*) was generated and placed on the chromosome of *M. maripaludis* JJ. Determining Bla activity under various growth conditions using the chromogenic substrate nitrocefin revealed the dependence of *frc* expression on selenium supply, on growth phase, and on nature and concentration of the energy substrate.
In order to identify factors involved in selenoprotein synthesis and in selenium-dependent gene regulation, the *M. maripaludis* strain carrying the reporter gene fusion was subjected to random mutagenesis using a modified insect transposable element. Over 6000 mutants were generated and screened for deregulated Bla activity. For those mutants exhibiting the desired phenotype, the transposon insertion site on the chromosome was mapped. The results of these efforts will be presented and discussed with respect to current knowledge about selenium biology in *Archaea*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

300/ARP
Structure and function of SAM-dependent methyltransferase HcgC involved in [Fe]-hydrogenase cofactor biosynthesis
L. Bai*[1], T. Wagner[1], T. Fujishiro[1], U. Ermler[2], S. Shima[1]
[1]*Max Planck Institute for Terrestrial Microbiolgy, Marburg, Germany*
[2]*Max-Planck-Institut für Biophysik, Frankfurt am Main, Germany*

[Fe]-hydrogenase is found in many hydrogenotrophic methanogenic archaea and produced under nickel limiting conditions. The iron in [Fe]-hydrogenase is part of the FeGP cofactor, where the redox-inactive low-spin Fe(II) is ligated to a Cys176-thiolate, two CO, the pyridinol N, and the acyl-C of the

acylmethyl substituent of pyridinol. Further pyridinol substituents are a guanosine monophosphate group and two methyl groups. Those methanogenic archaea containing the *hmd* genes ([Fe]-hydrogenase structural genes) also possess close besides on the genome *hmd*-co-occurring (*hcg*) genes (*hcgA–G*) which suggests their participation in cofactor biosynthesis [1]. To identify the biosynthetic precursors, we retrosynthetically analyzed the cofactor isolated from cells grown with stable-isotope-labeled substrates. This analysis indicated that a *S*-adenosylmethionine (SAM) dependent methyltransferase catalyzes the formation of the 3-methyl group of the pyridinol moiety. To analyze the function of the *hcg* genes, we employed a structure–to-function strategy [2,3]. We showed, first, that the crystal structure of HcgC resembles that of SAM-binding enzymes and, second, in co-crystallization studies, that SAM and its demethylated product, *S*-adenosylhomocysteine, can bind to HcgC. Accordingly, HcgC is a SAM-dependent methyltransferase. To find out the substrate of HcgC, enzyme reaction analysis was performed by using chemically synthesized pyridinol compounds as methyl acceptor. The reaction products were determined by MS and NMR. Based on these results, we confirmed that HcgC is a methyltransferase catalyzing the formation of the 3-methyl group of pyridinol [4]. Co-crystallization of HcgC and the pyridinol substrate was performed to analyze the catalytic mechanism.

References

[1] Bai L., Fujishiro T., Huang G., Koch J., Takabayashi A., Yokono M., Tanaka A., Xu T., Hu X., Ermler U. and Shima S. (2017) Towards artificial methanogenesis: biosynthesis of the [Fe]-hydrogenase cofactor and characterization of the semisynthetic hydrogenase. *Faraday Discuss*. In press.
[2] Fujishiro, T., Tamura, H., Schick, M., Kahnt, J., Xie, X., Ermler, U. & Shima, S. (2013) Identification of the HcgB enzyme in [Fe]-hydrogenase-cofactor biosynthesis. *Angew. Chem. Int. Ed*. 52: 12555–12558.
[3] Fujishiro, T., Kahnt, J., Ermler, U. & Shima, S. (2015) Protein-pyridinol thioester precursor for biosynthesis of the organometallic acyl-iron ligand in [Fe]-hydrogenase cofactor, *Nat. Commun*. 6: 6895.
[4] Fujishiro, T., Bai, L., Xu, T., Xie, X., Schick, M. Kahnt, J., Rother, M., Hu, X., Ermler, U. & Shima, S. (2016) Identification of HcgC as SAM-dependent pyridinol methyltransferase in [Fe]-hydrogenase cofactor biosynthesis. *Angew. Chem. Int. Ed.* 55: 9648−9651.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

301/ARP

Characterization of the putative Radical SAM methyl transferases responsible for the unique C-methylations of Gln and Arg in the methyl-coenzyme M reductase

D. Deobald*[1], M. Rother[2], L. Adrian[3], G. Layer[1]
[1]*University of Leipzig, Biochemistry, Leipzig, Germany*
[2]*TU Dresden, Dresden, Germany*
[3]*Helmholtz Centre for Environmental Research, Leipzig, Germany*

Biological methane formation is a process carried out by methanogenic archaea belonging to the phylum of *Euryarchaeota* (1). The methyl-coenzyme M reductase (MCR) is the key enzyme of this process and catalyzes the final reaction step. In this terminal step, methyl-coenzyme M and coenzyme B are converted to the heterodisulfide of coenzyme M and B with concomitant release of CH_4 (2). The crystal structures of MCRs from different methanogenic archaea revealed the presence of six modified amino acids near the active site of the α subunit (McrA). There are four methylated amino acids, one didehydroaspartate and one thiopeptide thioglycine (3), (4). Whereas the methylhistidine and thioglycine occur in all analyzed MCRs and seem to be essential, the importance of other modifications is less obvious, because they are not conserved in all MCRs. Nevertheless, the modifications 2-(*S*)-methylglutamine and 5-(*S*)-methylarginine are quite unique and have never been observed before (3). These methyl groups are introduced most probably co- or post-translationally by unknown Radical SAM methyl transferases (3), (5).

Our goal is to identify and characterize the Radical SAM methyl transferases responsible for the methylation of Gln and Arg in the McrA. For this purpose genome analysis and alignments of different methanogenic archaea were conducted. In close proximity of *mcrA* two different gene candidates encoding two Radical SAM enzymes were observed that might be responsible for the modifications 2-(*S*)-methylglutamine and 5-(*S*)-methylarginine.

We have produced one respective candidate protein Ma_4551 from *Methanosarcina acetivorans* C2A heterologously in *Escherichia coli* and are going to further characterize it and establish an enzyme activity assay, analyzing the methylation of Gln and Arg. Moreover, we have created a knockout strain by deleting the candidate gene *ma_4551* in *M. acetivorans* and aim to analyze the occurring phenotype and the modifications in the McrA of the mutant strain by LC-MS/MS.

References

[1] Thauer, R. K. (1998) Biochemistry of methanogenesis: a tribute to Marjory Stephenson. *Microbiol* 144, 2377–2406.
[2] Ellermann, J., Hedderich, R., Böcher, R. *et al.* (1988) The final step in methane formation. Investigations with highly purified methyl-CoM reductase (component C) from *Methanobacterium thermoautotrophicum* (strain Marburg). *Eur J Biochem* 172 (3), 669–677.
[3] Kahnt, J., Buchenau, B., Mahlert, F. *et al.* (2007) Post-translational modifications in the active site region of methyl-coenzyme M reductase from methanogenic and methanotrophic archaea. *FEBS J* 274, 4913–4921.
[4] Wagner, T., Kahnt, J., Ermler, U. *et al.* (2016) Didehydroaspartate Modification in Methyl-Coenzyme M Reductase Catalyzing Methane Formation. *Angewandte Chemie (International ed. in English)* 55 (36), 10630–10633.
[5] Buckel, W. & Thauer, R. K. (2011) Dual role of *S*-adenosylmethionine (SAM^+) in the methylation of sp^2-hybridized electrophilic carbons. *Angew Chem Int Ed* 50 (45), 10492–10494.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

302/ARP

Investigating the role of phosphatases in the archaellum regulatory network of *Sulfolobus acidocaldarius*

X. Ye*[1], S. V. Albers[1]
[1]*Albert-Ludwigs-Universität Freiburg, Freiburg, Germany*

Introduction: The archaellum, a type IV pilus like structure is essential for motility in Archaea. In the crenarchaeon *Sulfolobus acidocaldarius* the archaellum is synthesized under nutrient stress[1]. Two archaellum regulators (ArnA and ArnB) were phosphorylated in vitro, which suggested that protein phosphorylation may be involved in archaellum synthesis[2]. In *S. acidocaldarius*, only two phosphatases (Saci-PTP and Saci-PP2A) were found. Δ*saci_pp2a* showed hypermotility, while motility of Δ*saci_ptp* was almost the same as the wild type strain. Expression of ArnR and ArnR1, the positive regulators of the archaellum filament protein FlaB, were increased in the Δ*saci_pp2a* mutant[3]. Further, ArnB, ArnR and AbfR1 were phosphorylated in the phosphoproteome of the Δ*saci_ptp* mutant[3]. All these results verified the importance of reversible protein phosphorylation in the archaellum regulatory network.

Objective: In this study, we aim to investigate the cross-talk between both phosphatases and archaellum regulators. Also, new interaction partners of both phosphatases involved in motility are identified.
Methods: To analyze expression of archaellum regulators during archaellum synthesis, samples were prepared by starvation assay, and analyzed by qPCR and Western blot. Both phosphatases were tagged in the genome to facilitate pull down assays for the identification of further interaction partners.
Results: Results of qPCR and Western blot showed that the expression of the archaellum regulators in *Δsaci_ptp and Δsaci_pp2a* under starvation was similar to the wild type strain, which indicates that Saci-PTP and Saci-PP2A do not regulate the archaellum regulators at their expression level. *Saci_ptp* and *saci_pp2a* were successfully tagged in the genome and pull-down assays were performed. Although no specific enriched band was detected by silver staining, Western blot analysis using antibodies reacting against archaellum regulators showed that ArnA could interact with Saci-PP2A. Further, we explored the interaction between ArnA and Saci-PP2A at different time points during 4 h starvation, and observed that such interaction persisted over the time.
Conclusion: Taken together, we showed that both phosphatases affect motility not by regulating expression of archaellum regulators, but most probably by post translation modification. Archaellum expression is regulated by a sophisticated regulatory network in *S. acidocaldarius*. And more and more players are being identified.

References
[1] Lassak, K., Neiner, T., Ghosh, A., Klingl, A., Wirth, R. & Albers, S.V. Mol Microbiol, 83(2012) p110-124.
[2] Reimann, J., Esser, D., Orell, A., Amman, F., Pham, T.K., Noirel, J., Lindås, A.C., Bernander, R., Wright, P.C., Siebers, B. & Albers, S.V. Mol Cell Proteomics, 12 (2013) p3908-3923.
[3] Reimann, J., Lassak, K., Khadouma, S., Ettema, T.J., Yang, N., Driessen, AJ., Kling,l A. & Albers, S.V. Mol Microbiol, 86(2012) p24-36.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

303/ARP
New interaction partner and iCLIP analysis of RNA substrates of the archaeal exosome
S. Gauernack*[1], J. Bathke[1], O. Roßbach[1], L. Hou[1], G. Klug [1], E. Evguenieva-Hackenberg[1]
[1]*Institute for Micro- and Molecularbiology , Gießen, Germany*

Question: The exosome is a protein complex with major roles in degradation and processing of RNA in Archaea and Eukarya. In the Archaeon *Sulfolobus solfataricus* it is a phosphorolytic 3-5 exoribonuclease, which is also able to synthesize A-rich RNA-tails that are assumed to destabilize RNA. It is composed of a catalytically active hexameric ring with an RNA binding cap that comprises the eukaryotic orthologs Rrp4 and Csl4, as well as the archaea-specific subunit DnaG which needs Csl4 for its interaction with the exosome. Heterogeneous exosomal complexes were described in *S. solfataricus*, suggesting the existence of additional interaction partner which may influence the activity and substrate specificity of the complex [1, 2, 3]. The latter was analyzed *in vitro* with reconstituted exosomes, but global analysis of the exosomal substrates is not available yet.
The aim of our work was to analyze the interaction of the exosome with Nop5, a protein commonly known as part of an RNA methylation complex and that co-immunoprecipitated with the exosome from *S. solfataricus*. Another major goal was the transcriptome-wide analysis of substrates of the archaeal exosome.
Methods: The exosome was reconstituted *in vitro* using purified recombinant subunits or *in vivo* by co-expression of its subunits in *E. coli*. Protein-protein interactions were analyzed by coimmunoprecipitation of reconstituted protein complexes and bacterial two hybrid system. Global detection of archaeal RNA substrates was performed by iCLIP (individual-nucleotide resolution UV cross-linking and immunoprecipitation). For enzyme activity and substrate specificity tests, we used our established RNA degradation-, polyadenylation- and EMSA-assays.
Results: *In vitro*, Nop5 was co-immunoprecipitated with the Rrp4-exosome but neither with the Csl4-exosome nor the catalytically active hexameric ring of the complex, to which the Rrp4- or the Csl4-RNA- binding cap is attached in the reconstituted complexes. Furthermore, Rrp4 and Nop5 showed an interaction in a bacterial two-hybrid system. Moreover, Nop5 had no influence on RNA degradation by the reconstituted Rrp4-exosome, but increased the polyadenylation of RNAs by this complex. The iCLIP analysis revealed numerous natural substrates of the exosome in *S. solfataricus*. Among them were RNAs corresponding to repetitive sequences, small RNAs as well as 5- and 3-regions of mRNAs.
Conclusions: Our data suggest that Nop5 modulates the polyadenylation activity of the archaeal exosome. Furthermore, for the first time transcriptome-wide detection of exoribonuclease substrates was achieved by iCLIP in *S. solfataricus*.

References
[1] Evguenieva-Hackenberg E et al. EMBO Rep. 2003 4(9):889-93.
[2] Witharana et al. Biochimie 2012 94: 1578-1587.
[3] Evguenieva-Hackenberg E et al. Rev RNA. 5(5):623-35

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

304/ARP
The end is in sight - identification of the *Haloferax volcanii* transcription termination sites by dTermSeq
L. K. Maier*[1], S. J. Berkemer[2], F. Pfeiffer[3], P. F. Stadler[2], A. Marchfelder[1]
[1]*Ulm University, Biology II, Ulm, Germany*
[2]*Leipzig University, Bioinformatics Group, Leipzig, Germany*
[3]*Max-Planck-Institute of Biochemistry, Computational Biology Group, Martinsried, Germany*

Understanding the transcriptional landscape is key to unravelling the full genetic potential of an organism. Regulation and tight control of transcription as well as its adaptation to varying environmental conditions are the foundation for the plethora of microbial life.
We are interested in unravelling the transcriptional landscape of *Haloferax volcanii*, a model organism for halophilic Archaea. The genome of *Haloferax* is sequenced and it is genetcially tractable (1). Recently, transcription start sites of *H. volcanii* have been determined using a differential RNA-Seq approach (2). In the study presented here, we want to complete the picture of the transcriptional landscape of *H. volcanii* by a complementary analysis of transcription termination sites.
Only a limited body of evidence exits as to the motifs and structures governing transcription termination in the archaea but highlights an involvement of poly-U stretches (3). A seminal study by Dar et al. introduced Term-Seq as sequencing method for the transcriptome-wide mapping of RNA 3"-ends that was shortly after applied for two archaeal organisms *Methanosarcina mazei* and *Sulfolobus acidocaldarius* (3, 4). The analysis by Dar et al. supports the key role of poly-U traces as archaeal termination signals but also points at informational content on both sequence and structural level that might govern efficiency of termination. It also uncovered use of multiple termination sites resulting in alternative 3" UTRs with regulatory potential and a strong

resemblance to eukaryotic non-coding RNA-based systems. But the analysis also revealed interspecies differences between the two model archaea studied calling for further investigations to discern common features and species-specific peculiarities. Therefore, we want to determine the transcription termini in the haloarchaeon *H. volcanii*. TermSeq, the method used by Dar et al., is based on a total RNA fraction that contains a mixture of RNA 3´ ends generated by processing and transcription termination. We modified this approach and used a differential sequencing method (analogous to the dRNA-Seq approach for the termination of TSS sites), which we termed dTerm-Seq, to determine the transcription termination sites (TTS). One RNA fraction is enriched for primary transcripts using terminator exonuclease-(TEX)-treatment, while the other RNA fraction contains all RNAs (processed RNAs and primary transcripts). Comparison of both libraries will identify on one hand RNA 3´ ends produced by transcription termination and on the other hand RNA 3´ ends generated by processing. We will combine the dTerm-Seq analysis with *in vivo* reporter systems to confirm identified termination signals.

References
(1) Hartman et al., 2010. PloS one 5, e9605.
(2) Babski et al., 2016 BMC Genomics 17.
(3) Dar et al., 2016b. Nature Microbiology 1, 16143.
(4) Daret et al., 2016a. Science 352, aad9822-aad9822.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

305/ARP

Bacterial-like ABC-transporter for L-arabinose and D-xylose in *S. acidocaldarius*

N. J. H. van der Kolk*[1], M. Wagner[1], A. Wagner[1], S. V. Albers[1]
[1]*Albert-Ludwigs-Universität Freiburg, Biologie II, Freiburg, Germany*

Introduction: The thermophilic archaeon *S. acidocaldarius* can utilize different carbon sources, including the pentoses D-xylose and L-arabinose. For the uptake of sugars membrane transport proteins are essential, since sugars cannot cross the cell membrane by diffusion. We found the ABC transporter involved in the uptake of the pentoses of which the sugar binding protein (SBP) is novel to Archaea. This ABC-transporter belongs, according the Transporter Classification Database (TCDB), to the CUT2 family. In addition we found an Ara-box that is located in the promotor region of arabinose induced genes.
Objective: The transport, regulation and utilization of sugars in *S. acidocaldarius* is poorly understood. In the study we aimed to identify and characterize transport proteins responsible for the uptake of L-arabinose and D-xylose.
Methods: RNA sequencing data revealed three highly upregulated genes encoding a so far unknown and uncharacterized ABC transport system. To confirm the role of the transport system a deletion mutant was monitored on different carbon sources. A quantitive RT-PCR was used to examine transcriptional levels of the SBP. The presence and function of the Ara-box was tested with an ONPG essay.
Results: The transcriptional levels of the transporter operon were induced upon the presence of L-arabinose and D-xylose. When the Ara-box was deleted the growth on the pentose substrate was strongly reduced. Also, deletion of the Ara-box caused a significant decrease in the b-galactosidase activity.
Conclusion: Taken together our results deepen the knowledge about this unique transport system for L-arabinose and D-xylose in *Sulfolobus acidocaldarius*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

306/ARP

Flagella of *Methanocaldococcus villosus* co-localize with a polar cap and play a crucial role in surface colonization

A. Bellack*[1]
[1]*University of Regensburg, Institute of Microbiology, Regensburg, Germany*

Introduction: The hyperthermophilic Archaeon *Methanocaldococcus villosus* was isolated from a shallow submarine hydrothermal system at Kolbeinsey Ridge, north of Iceland. Its up to 50 polar flagella were shown to be multifunctional organelles involved in motility, adhesion to surfaces, and formation of cell-cell contacts [1]. Swimming studies identified *M. villosus* to be the fastest organism on earth known so far. Apart from these very rapid relocation movements, cells exhibited a much slower seek movement which initializes adhesion [2].
Objectives: Up to now, it is only poorly understood how archaeal flagella are anchored in the cell and how their fast and coordinated rotation is achieved without disrupting the cell. Choosing *M. villosus* as a model organism, we aim to investigate the surface attachment of extremophilic Archaea with focus on the role of cell appendages. Furthermore, we want to compare the cell architecture of planktonic and adherent cells.
Material & Methods: To determine the cellular ultrastructure of *M. villosus*, cells were prepared for electron microscopy by high-pressure freezing/freeze substitution or conventional chemical fixation [3]. Specimens were investigated by transmission electron microscopy or by focused ion beam scanning electron microscopy with regard to substructures in the cell and anchoring of flagella in the membrane. Adhesion and biofilm formation was tested by adding various materials, e.g. gold grids or glassy carbon, to culture media. After growth, surfaces were analyzed using different microscopic techniques.
Results: Independent from the preparation method used, electron microscopic analyses of *M. villosus* revealed a well-preserved cell envelope and that flagella were anchored in close proximity to a polar cap. In some cases, the polar cap co-localized with a complex structure resembling bacterial chemoreceptor arrays. A comparison of planktonic to adherent cells showed that the number of flagella and pili increases when cells are attached to a surface, associated with a change to a peritrichous arrangement. In addition, the formation of microcolonies and biofilms correlated with production of extracellular polymeric substances.
Conclusion: Altogether, these results demonstrate the crucial role of cell appendages in the surface colonization of *M. villosus* and broaden our knowledge of (eury-)archaeal biofilm formation. The ultrastructural analyses of this study proved the existence of a polar cap and a chemosensory array in close proximity to flagella confirming the suggested connection of the chemotactic system with the flagellar apparatus.

References
[1] A. Bellack et al. (2011) IJSEM 61:1239-1245
[2] Herzog & Wirth (2012) AEM 78:1670-1674
[3] R. Rachel et al. (2010) in Methods in Cell Biology: Electron microscopy of model systems 47-69

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

307/ARP

Requirement of the energy-converting methyltransferase MTR in *Methanosarcina acetivorans*

C. Schöne*[1], M. Rother[1]
[1]*TU Dresden, Institut für Mikrobiologie, Dresden, Germany*

Methanogenesis is an important part of the global carbon cycle and exclusively carried out by methanogenic members of the domain *Archaea*. While most methanogens employ only a single methanogenic pathway, *Methanosarcina* (*M.*) species are more metabolically versatile. *M. acetivorans* cannot use hydrogen and carbon dioxide as growth substrates due to the lack of a functional hydrogenase system, but is able to grow on methylated compounds (e.g. methanol), acetate or carbon monoxide (CO). Common to methanogenesis from any of these substrates is involvement of the energy- converting N^5- methyl-tetrahydrosarcinapterin (H_4SPT): coenzyme M (HS-CoM) methyltransferase (MTR), which couples the reversible methyl-group transfer from H_4SPT to HS-CoM to translocation of sodium ions, and, thus, serves as a chemiosmotic coupling site. However, previous genetic and biochemical analyses of *M. barkeri* and *M. acetivorans* led to the proposal of a cytoplasmatic methyl-transfer from H_4SPT to CoM (and *vice versa*) not involving MTR. Also, *M. acetivorans* produces during CO-dependent growth substantial amounts of acetate, which is generated through a pathway analogous to that found in acetogenic bacteria and could, thus, be coupled to energy conservation by substrate-level phosphorylation. In order to address the requirement of MTR for CO-dependent growth in *M. acetivorans* and to shed more light on the potential methyl-transfer bypass, the encoding operon, *mtrEDCBAFGH* was deleted from the chromosome. Phenotypic analysis of the *mtr* mutant will be presented, including the nature and amounts of the metabolites produced from various growth substrates. The data will be used to discuss a possible MTR-bypass in *M. acetivorans* and the generally accepted unconditional requirement of methane formation for energy conservation in methanogens.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

308/ARP

Biofilm formation of *Halobacterium salinarum* R1

G. Losensky*[1], K. Jung[2], H. Urlaub[3,4], F. Pfeifer[1], C. Lenz[3,4], S. Fröls[1], G. Losensky[1]
[1]*TU Darmstadt, Microbiology and Archaea, Darmstadt, Germany*
[2]*University of Veterinary Medicine Foundation, Institute for Animal Breeding and Genetics, Hannover, Germany*
[3]*Max Planck Institute for Biophysical Chemistry, Bioanalytical Mass Spectrometry Group, Göttingen, Germany*
[4]*University Medical Center Göttingen, Institute of Clinical Chemistry, Bioanalytics, Göttingen, Germany*

Introduction: Knowledge about biofilms, *i.e.* multicellular microbial communities, is still limited, especially in Archaea. Among the haloarchaeal strains tested to date, the extremely halophilic euryarchaeote *Halobacterium salinarum* R1 shows one of the strongest adhesion and biofilm formation capabilities [1]. However, little is known about the underlying processes contributing to the sessile lifestyle of this species.
Objectives: The present study focuses on the phenotypic and proteomic differentiations during biofilm formation of *Hbt. salinarum* R1.
Materials & methods: Light microscopy, confocal laser scanning microscopy (CLSM) as well as a fluorescence-based adhesion assay were used to monitor and quantify the development of biofilms over time. SDS-PAGE of cell lysates prepared from planktonic and sessile cells was performed to test for a potential differentiation between the respective proteomes. Relative quantification of proteins in planktonic cells, initial biofilms and mature biofilms was done by use of a label-free mass spectrometric SWATH-LC/MS/MS analysis.
Results: During 15 days of growth, a steady increase of adherent cells and the formation of mature/complex biofilms up to 25 µm thick was observed on solid plastic and glass surfaces. Moreover, the production of a biofilm matrix containing extracellular DNA (eDNA) and glycosidic components was detected.
A comparison of protein samples obtained from planktonic and biofilm cells revealed fundamentally different protein patterns under both conditions. In our proteomic approach 63.2 and 58.6% of the predicted *Hbt. salinarum* R1 proteome could be detected and quantified, respectively
The relative quantification yielded between 55 and 245 proteins strongly altered (> 2-fold) when two of the cellular states were compared. 882 proteins showed statistically significant abundance changes, correspoding to 60.8% of the quantified proteins and 34.2% of the total proteome, respectively. The relative changes detected ranged between 195-fold increase of an uncharacterized glutamine-rich alkaline protein and 22.8-fold decrease of ribonucleoside-diphosphate reductase subunit beta. The most striking effects were observed with proteins involved in energy conversion, as well as proteins acting in nucleotide-, amino acid- and lipid metabolism. In addition, proteins associated with protein biosynthesis and cellular processes like cell motility and signal transduction were strongly affected.
Conclusion: This work represents the first comprehensive description of haloarchaeal biofilm formation using the example of *Hbt. salinarum* R1 [2]. The high diversity of the cellular processes affected reflects the fact, that biofilms represent a distinct cellular state.

References

[1] Fröls S, Dyall-Smith M, Pfeifer F. Environ Microbiol. 2012 Dec;14(12):3159-74.
[2] Losensky G, Jung K, Urlaub H, Pfeifer F, Fröls S, Lenz C. Proteomics. 2016 Sep 8. [Epub ahead of print]

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

309/ARP

Laboratory cultivation of acidophilic nanoorganisms. Physiological and bioinformatic dissection of a stable laboratory co-culture.

S. Krause*[1], A. Bremges[1,2], P. C. Münch[2], A. C. McHardy[2], J. Gescher[1]
[1]*Karlsruhe Institute of Technology, Applied Biology, Karlsruhe, Germany*
[2]*Helmholtz Centre for Infection Research, Computational Biology of Infection Research, Braunschweig, Germany*

To date only two ultra-small archaea (*Nanoarchaeum equitans* and the recently cultured *Nanopusillus acidilobi)* were cultivated and characterized under laboratory conditions. Both are characterized by reduced genomes (~0.5 kbp and 0.6 kbp, respectively) lacking nearly all genes essential for primary biosynthetic functionalities (Waters *et al.*, 2003; Wurch *et al.*, 2016), which indicate strong dependencies on their hosts. Compared to *N. equitans* and *N. acidilobi*, the genome of the members of the not yet isolated ARMAN (Archaeal Richmond Mine Acidophilic Nanoorganisms) is with 1 Mbp twice as big and contains key functions of a central carbon metabolism (Baker *et al.*, 2010), which is indicative for a more independent lifestyle.
This study describes an enrichment process to culture ARMAN and a corresponding physiological, metagenomic and metatranscriptomic analysis (Krause *et al., submitted*). After 2.5 years of successive transfers in an anoxic medium containing ferric sulfate as an electron acceptor, a consortium was attained that is comprised of two members of the *Thermoplasmatales*, an ARMAN, as well as a fungus. Growth of all archaea was dependent on ferric iron reduction as was revealed by ferrous iron quantification and qPCR based cell quantification. From this

culture, we isolated the fungus and one of the members of the *Thermoplasmatales* in pure culture. While the fungus shows an ITS region identity of 99% to *Acidothrix acidophila*, the 16S rRNA identity of the archaeon is only 91.6% versus *Thermogymnomonas acidicola* as the most closely related microorganism. Hence, the isolate is most likely the first member of a new order within the *Thermoplasmatales*. The ARMAN-like microorganism seems to be incapable of sugar metabolism because the key genes for sugar catabolism and anabolism could not be identified. Transcriptomic analysis suggests that the TCA cycle funneled with amino acids is the main metabolic pathway used by the archaea of the community. Microscopic analysis revealed that growth of the ARMAN is supported by the formation of cell aggregates. These might enable cross feeding of sugars produced by other community members to the ARMAN.

References

[1] Baker BJ, Comolli LR, Dick GJ, Hauser LJ, Hyatt D, Dill BD, *et al.* (2010). Enigmatic, ultrasmall, uncultivated Archaea. *Proc Natl Acad Sci U S A* 107: 8806–8811.
[2] Waters E, Hohn MJ, Ahel I, Graham DE, Adams MD, Barnstead M, *et al.* (2003). The genome of Nanoarchaeum equitans: insights into early archaeal evolution and derived parasitism. *Proc Natl Acad Sci U S A* 100: 12984–8.
[3] Wurch L, Giannone RJ, Belisle BS, Swift C, Utturkar S, Hettich RL, *et al.* (2016). Genomics-informed isolation and characterization of a symbiotic Nanoarchaeota system from a terrestrial geothermal environment. *Nat Commun* 7: 1–10.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Cyanobacteria (FG CB)

310/CBP
Unexpected capacity for organic carbon assimilation by *Thermosynechococcus elongatus*, a crucial photosynthetic model organism

Y. Zilliges*[1], H. Dau[1]
[1]*Freie Universität Berlin, 14195, Germany*

Genetic modification of key residues of photosystems is essential to identify functionally crucial processes by spectroscopic and crystallographic investigation; the required protein stability favors use of thermophilic species. The currently unique thermophilic model organism is the cyanobacterial genus *Thermosynechococcus* which has been known as an obligate autotrophic photosynthesizer as yet. In depth physiological and biochemical analysis reveals that *T. elongatus* is indeed able to assimilate organic carbon, specifically D-fructose. This is in line with the observation that most photosynthetic microorganisms seem to have the capacity for both inorganic (by photosynthesis) and organic (by respiration) carbon assimilation, possibly to overcome inorganic macronutrient deficiencies. However, D-fructose assimilation by *T. elongatus* originates (under certain conditions) a bleaching phenotype that is similar as in the Δ*pmgA*-mutant (photomixotrophic growth-sensitive) of *Synechocystis* sp. PCC 6803. The simultaneous activity of photosynthetic and respiratory reactions in both the same membrane and cytosolic compartment have been monitored in this study as well in order to understand (and to manipulate) both this particular phenotype (i) and the regulation and mechanism of this crucial (and useful) metabolic capacity (ii). Only the ability of *T. elongatus* to grow in the presence of both a photosynthesis inhibitor and an organic carbon source opens the door towards crucial amino acid substitutions in photosystems by the rescue of otherwise lethal mutations.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

311/CBP
Assessing the potential of formate assimilation in the cyanobacterial model *Synechocystis* sp. PCC 6803.

M. Hagemann[1], S. Song[1], E. M. Brouwer*[1]
[1]*University Rostock, Plant Physiology, Rostock, Germany*

Cyanobacteria are increasingly being used as cell factories for the biotechnological production of various organic compounds and as models for the plant-type carbon assimilation. Photosynthetic carbon assimilation in cyanobacteria as well as plants depends on the activity of RubisCO, an enzyme with relatively low activity and affinity toward the substrate CO_2. To promote the photosynthetic assimilation of inorganic carbon artificial pathways have been modelled *in silico*, which can operate in addition to the activity of RubisCO. One possibility is the reduction of CO_2 to formate, which can subsequently be incorporated into the carbon metabolism. To assess this possibility, we will use the cyanobacterial model strain *Synechocystis* sp. PCC 6803. This cyanobacterium can take up formate and formate supplementation improved growth of the wild type under specific growth conditions. This result indicates that *Synechocystis* has the principal capability for formate assimilation. Strains expressing heterologous enzymes for formate synthesis and assimilation are currently used to achieve an efficient formate assimilation pathway working in parallel to the photosynthetic CO_2 assimilation. Furthermore, mutations of genes encoding for formate assimilation enzymes will be generated to establish a strain, which is dependent on formate for the synthesis of precursors for purine biosynthesis. Such mutant strains will be used as hosts to select for efficient formate synthesis enzymes in the cyanobacterial cell.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

312/CBP
Identification of µ-Proteins and Non-Coding RNAs in Cyanobacteria and Functional Assignments to Them

W. Hess*[1], D. Baumgartner[1], M. Kopf[1], S. Klähn[1], C. Steglich [1]
[1]*University of Freiburg, Freiburg, Germany*

Background: Transcriptomic analyses yielded insight into the complex composition of cyanobacterial transcriptomes. The recent functional characterization of several short mRNAs and non-coding RNAs has revealed new mechanisms and surprising insight into construction principles of the regulatory system. These include the roles of transcription factors, sRNAs and the fact that some transcripts previously considered non-coding encode proteins ≤80 amino acids (1). Such µ-proteins are a systematically underestimated class of gene products in bacteria. However, photosynthetic cyanobacteria provide a paradigm for small protein functions due to extensive work on the photosynthetic apparatus. This has led to the functional characterization of 19 very small proteins ≤50 amino acids. The shortest annotated protein conserved in cyanobacteria is with 29 amino acids the cytochrome b6-f complex subunit VIII, encoded by *petN*. In analogy, previously unstudied small ORFs with similar degrees of conservation might encode µ-proteins of high relevance in other functional contexts. **Results:** We used comparative transcriptomic information available for two model cyanobacteria, *Synechocystis* sp. PCC 6803 and PCC 6714 (2,3) for the prediction of small ORFs. We found 293 transcriptional units containing candidate small ORFs ≤80 codons in *Synechocystis* sp. PCC 6803, including the mRNAs encoding known small proteins of the photosynthetic apparatus (4). From these transcriptional units, 146 are shared between the two strains, 42 are shared with the plant *A. thaliana* and 25 with *E. coli*. To

verify the existence of the respective µ-proteins *in vivo*, we selected five genes to which a FLAG tag sequence was added and re-introduced them into *Synechocystis* sp. PCC 6803. These were the previously annotated gene *ssr1169*, two newly defined genes *norf1* and *norf4*, as well as *nsiR6* and *hliR1* (for nitrogen stress-induced RNA 6 and high light inducible RNA 1), which originally were considered non-coding). Upon activation of expression from the native promoters, all five proteins were detected. The regulation of their expression and of further candidates provides intriguing hints towards functions in the responses to nitrogen and carbon starvation.

References

[1] Kopf M., Hess W.R. (2015) Regulatory RNAs in photosynthetic cyanobacteria. FEMS Microbiol. Rev. *39*, 301–15.
[2] Kopf M., Klähn S., Scholz I., Matthiessen J.K.F., Hess W.R., Voß B. (2014) Comparative analysis of the primary transcriptome of *Synechocystis* sp. PCC 6803. DNA Research *21,* 527-539.
[3] Kopf M., Klähn S., Scholz I., Hess W.R., Voß B. (2015) Variations in the non-coding transcriptome as a driver of inter-strain divergence and physiological adaptation in bacteria. Scientific Reports *5,* 9560, DOI: 10.1038/srep09560.
[4] Baumgartner D., Kopf M., Klähn S., Steglich C., Hess W.R. (2016) Small proteins in cyanobacteria provide a paradigm for the functional analysis of the bacterial micro-proteome. BMC Microbiology*, in press.* DOI: 10.1186/s12866-016-0896-z.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

313/CBP

Cyanobacterial Photosynthesis: from Basic Research to Solar Technology

M. Nowaczyk*[1]
[1]*Ruhr-University Bochum, Bochum, Germany*

Photosynthesis is the most fundamental process for life on earth and cyanobacteria are of particular importance. They "invented" oxygenic photosynthesis ~3.5 billion years ago by using water as an unlimited electron source in the conversion of light energy into chemical energy. Photosystem II (PSII), nature"s water splitting catalyst, is of special interest as it is the only enzyme that catalyse the light-driven oxidation of water.
We have shed light on the biogenesis and assembly of this multisubunit membrane protein complex by chromatographic separation of distinct assembly intermediates [1]. Most structural variations of the corresponding PSII species are based on a different set of protein factors bound to the luminal interface of the complex. We have analysed the interaction between PSII and its soluble binding partners by surface plasmon resonance (SPR) spectroscopy and a combination of chemical cross-linking with mass spectrometry (CX-MS). This approach revealed a network of assembly factors (e.g. Psb27, Psb28, Psb32, CyanoQ, CyanoP etc.) that assist PSII maturation in a well concerted process [2].
Moreover, cyanobacterial photosynthesis serves as a toolbox for solar technology. Highly active photosystems can be integrated into artificial redox environments that mediate electron transfer *in vitro* [3]. We have developed biophotoelectrodes with immobilized photosystems that convert light energy into electricity and we are currently engineering electron pathways within and between the biological and chemical components for tailor-made applications [4]. This knowledge drives also the development of biotechnological innovations for whole-cell processes *in vivo* [5]. We are currently engineering cyanobacterial strains by re-routing of photosynthetic electron pathways towards recombinant oxidoreductases that catalyse the electron dependent conversion of bulk and fine chemicals [6]. Both concepts are part of a fast developing, fascinating research field at the interface between chemistry and biology.

References

[1] Heinz S, Liauw P, Nickelsen J, Nowaczyk M (2015) BBA Bioenergetics 1857:274-287
[2] Cormann KU, Möller M, Nowaczyk MM (2016) Front Plant Sci 7:157
[3] Nowaczyk MM and Plumeré N (2016) Nature Chemical Biology 12:990-991
[4] Kothe T, Plumeré N, Badura A, Nowaczyk MM, Guschin DA, Rögner M, Schuhmann W (2013) Angew Chem Int Ed 52:14233-14236
[5] Nowaczyk MM and Kourist R (2016) Biospektrum 22:765
[6] Köninger K, Gómez Baraibar A, Mügge C, Paul CE, Hollmann F, Nowaczyk MM, Kourist R (2016) Angew Chem Int Ed 55: 5582-5585

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Diagnostic Microbiology (StAG DV)

314/DVP

Killing kinetics of a bacteriophage endolysin with anti-*Staphylococcus aureus* activity

N. Schleimer*[1], U. Kaspar[1], D. Knaack[1], C. von Eiff[1,2], A. S. Scherzinger[3], H. Grallert[3], G. Peters[1], E. A. Idelevich[1], K. Becker[1]
[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*
[2]*Pfizer Pharma GmbH, Berlin, Germany*
[3]*Hyglos GmbH - a bioMérieux company, Bernried, Germany*

Question: S*taphylococcus aureus* represents one of the most serious pathogens worldwide. Chronic and persistent infections due to small-colony variants (SCVs), a slow growing subpopulation of *S. aureus*, complicate the therapy of *S. aureus* infections. Alternative prophylactic and therapeutic options are warranted to combat antimicrobial resistance. While previous studies have shown that recombinant bacteriophage endolysins are highly active against *S. aureus*, the activity of phage endolysins has not yet been systematically examined for SCVs. This study evaluates the *in vitro* activity of the endolysin HY-133 against clinical wild type (WT) *S. aureus* and their clonally identical SCVs in comparison to the beta-lactam antibiotic oxacillin at various growth phases.
Methods: A set of 12 clinical *S. aureus* strain pairs consisting of WTs and corresponding SCVs was chosen to evaluate the minimum inhibitory and bactericidal concentrations (MICs and MBCs) of endolysin HY-133 (Hyglos GmbH – a bioMérieux company, Bernried, Germany) and oxacillin (Sigma-Aldrich Co. LLC, Darmstadt, Germany). For all isolates, the direct colony suspension method was performed as recommended by CLSI. Additionally, the activities of both substances were evaluated during logarithmic growth of WTs and SCVs. Therefore MICs and MBCs were determined after 3 h of incubation in liquid medium. To further examine killing kinetics of HY-133 in comparison to oxacillin, time-kill curves were performed.
Results: For HY-133, 95.8% (n=46/48) of strains showed identical values for MIC and MBC. In detail, median MIC/MBC_{50} values of stationary growth cultures were 0.12 mg/L for WTs and 0.25 mg/L for SCVs, respectively. Comparable values were measured for cultures of logarithmic growth phase. Calculated medians of MIC/MBC_{90} were found to be 0.5 mg/L for all strain pairs and growth phases.
For oxacillin, 20.8% (n=10/48) of MBCs were higher than MICs. MIC/MBC_{50} values were 0.5 mg/L for WTs and 0.25 mg/L for SCVs for both growth phases. For all strain pairs MIC/MBC_{90} values were 1.0 mg/L with one exception for MIC_{90} tested from logarithmic growth (0.5 mg/L).

Time-kill studies revealed an eradication of 99.9% of the initial inoculum of all strains after 1 h of incubation with 4 mg/L HY-133. However, for all HY-133 concentrations a regrowth phenomenon was detected. In contrast, 4 mg/L of oxacillin eradicated 99.9% of the bacteria only after 4-24 h depending on the strain tested. For oxacillin, no regrowth was observed.
Conclusions: This study highlights the activity of HY-133 against *S. aureus* WTs and SCVs under different growth conditions. Although SCVs are known to show decreased susceptibilities to several antibiotics, HY-133 eradicates SCVs in the same extent as WTs. Compared to oxacillin, HY-133 shows a faster mode of action. However, regrowth phenomenon remains to be further elucidated.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

315/DVP
Evaluation of the Carbapenem Inactivation Method (CIM) for the detection of carbapenemase activity in Enterobacteriaceae in a routine laboratory
S. Engels-Schwarzlose*[1], A. Gehnen[1], A. Helmers[1], A. Helmer[1], R. Geisel[1], N. Pfennigwerth[2], B. Thoma[1]
[1]*Medizinische Laboratorien Düsseldorf, Mikrobiologie, Düsseldorf, Germany*
[2]*NRZ für Gram-negative Krankenhauserreger, Bochum, Germany*

Introduction: The rapid worldwide dissemination of carbapenemases has led to an increase in the prevalence of carbapenem-resistant Enterobacteriaceae and poses a significant threat to healthcare systems. Carbapenemase producing Enterobacteriaceae (CPE) carry the carbapenemase genes on mobile genetic elements and the resistance can spread through either clonal expansion or transfer of the carbapenemase genes to other bacteria. The fast and reliable detection of CPE is of great importance for treatment and implementation of infection control measures to avoid potential outbreaks and lateral spread of resistance. Phenotypic test methods like the modified Hodge Test (MHT) and the novel Carbapenem Inactivation Method (CIM) are simple and low-cost methods to identify CPE in the microbiology laboratory.
Objective: To establish the CIM in a routine laboratory this test was comparatively evaluated using a collection of Enterobacteriaceae isolates.
Methods: 113 Enterobacteriaceae isolates from clinical specimens collected between 2014-2016 and indicative for carbapenem-resistance were included in this study. The mean inhibitory concentration was determined by Vitek®2 (bioMérieux, France) and confirmed by gradient diffusion test. The MHT was performed as described in CLSI-document M100-21 (Clinical & Laboratory Standards Institute, USA). Briefly, for the CIM a meropenem disk was incubated in a suspension of the bacterial isolate and water. This meropenem disk was transferred on a Mueller-Hinton agar plate inoculated with a susceptible *Escherichia coli* strain. After a second incubation step carbapenemase activity could be detected by the absence of an inhibition zone. The results were verified by molecular detection of genes coding for carbapenemases.
Results: From the 113 investigated strains 64 were carbapenemase-producing isolates (including OXA- [n=36], NDM- [n=14], VIM- [n=8], and GIM-type [n=6]). All PCR-positive strains showed carbapenemase activity by CIM (sensitivity=100 %), while MHT failed in the detection of one NDM-carbapenemase.
In both phenotypic methods we obtained false positive results (MHT [n=18] and CIM [n=6]). While we could not detect a specific pattern for the MHT, the false positive results derived from the CIM were restricted to the genus *Enterobacter* (*E.*) [*E. aerogenes* (n=1) and *E. cloacae* complex (n=5)].
Conclusion: In our study the CIM showed a diagnostic performance of 100% negative predictive value and 91% positive predictive value. The CIM-test is easy to perform, cost-effective, highly robust, and easy to read. It is a reliable screening method that may be integrated in an algorithm intended to detect carbapenmases in Enterobacteriaceae in a routine laboratory. As a phenotypic method CIM may also detect carbapenemase activity even in strains with so far unknown carbapenemase encoding genes.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

316/DVP
Retrospective and Prospective Evaluation Study for the Simultaneous Detection of Seven Sexually Transmitted Pathogens
N. Wendt[1], J. Tittelbach[2], M. O. Grimm[3], C. Scheungraber[4], B. Löffler[1], M. Baier[1], M. Karrasch*[1]
[1]*Institute of Medical Microbiology, Jena, Germany*
[2]*Department of Dermatology, Jena University Hospital, Jena, Germany*
[3]*Department of Urology, Jena University Hospital, Jena, Germany*
[4]*Department of Gynecology, Jena University Hospital, Jena, Germany*

Background: Fast and accurate diagnosis of sexually transmitted infections (STI) is capable of preventing spread and severe complications of curable STIs through pathogen adapted antibiotic treatment regimens. Limitations of current STI diagnostic tools are the lack of simultaneous pathogen detections and quantification of results.
Materials/Method: All samples were processed with multiplex real-time PCR assay AnyplexTM II STI-7 (Seegene, Seoul, Korea), using thermal cycler CFX96TM (Bio-Rad Laboratories, Hercules, USA). The AnyplexTM II STI-7 detection assay covers Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Mycoplasma genitalium (MG), Mycoplasma hominis (MH), Ureaplasma parvum (UP), Ureaplasma urealyticum (UU) and Trichomonas vaginalis (TG) and is capable of detecting STI coinfections within one single run. This assay was used for a retrospective internal evaluation of known STI specimens (n=74) followed by a prospective analysis (January-November 2016) of clinical specimens (n=72, from 61 symptomatic patients) from different sites of urological, dermatological, gynaecological infections. Study samples (urethral/endocervical/rectal/oral swab samples; urine; intraoperatively processed ascites) were first tested using conventional gold standard methods, only for pathogen detection requested by clinicians. STI-7 results were compared to standard methods and transferred to the attending physician for treatment adjustment.
Results: Reported medical issues were urethritis, vaginitis, fever of unknown origin, screening/monitoring, and ovarian abscess. Among the tested samples, 27 of 72 (38%) were positive for at least one pathogen. AnyplexTM II STI-7 detected CT in nine (13%), NG in nine (13%), UP in twelve (17%), MG in three (4%), MH in five (7%), UU in four (6%) and TV in none of the analysed samples. STI coinfection was detected in ten samples, double infections in five (UU+UP; UU+CT; UP+CT; MH+UP; NG+UP), triple infections in three (2xCT+MH+UP; CT+NG+UU) and fourfold infections in two samples (CT+MH+MG+UP; CT+MG+UP+UU). It missed one CT (vaginal swab) and one NG (urine) infection. Additional nine single infections and nine out of ten multiple infections were detected using STI-7 panel. Compared to performed standard method results the assay achieved sensitivities from 88% to 100%, and specificities of 100%, with negative predictive values from 98% to 100%, and positive predictive values of 100%.
Conclusion: The AnyplexTM II STI-7 assay can easily be introduced into the microbiological laboratory work flow due to its short hands-on-time and the mutiplexicity of the PCR. The simultaneous detection of STI related pathogens provides a

comprehensive profile for each patient, enabling the clinician to decide on the best treatment options, thus decreasing antibiotic misuse and the risk of the infection spreading. Semi-quantitative results might be useful to determine disease severity and might enable clinicians to gain a complete package of diagnostic information including disease diagnosis, degree of disease severity, and monitoring of treatment. Further clinical studies on this topic are needed.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

317/DVP

Isolation of single stranded DNA aptamers for therapeutic and diagnostic applications

D. Kubiczek*[1], N. Bodenberger[1], T. Oswald[1], A. Wittgens[1], F. Rosenau[1]
[1]*Ulm University, Center for Peptide Pharmaceuticals, Ulm, Germany*

Aptamers are single stranded polynucleotides, which are able to bind specific targets with a high specificity. This is mediated by their three dimensional structure depending on the sequence of the aptamer. The high specificity of these molecules even allows the discrimination of the same target molecule with different conformations. The possible targets against which aptamers [A1] can be developed range from single molecules, like small organic compounds or proteins to whole mammalian or bacterial cells. In comparison to antibodies, aptamers are more stable against biodegradation and they exhibit no intrinsic toxicity or immunogenicity, which makes them a suitable agent for therapeutic use. Furthermore aptamers can be easily synthesized and chemically modified, for example with fluorescent dyes, making them promising agents in diagnostics. Fluorescently labelled aptamers have been used to rapidly detect the pathogenic *Pseudomonas aeruginosa* by fluorescence in situ hybridization. Other approaches make use of inhibitory effects of aptamers. Besides the prominent example of the FDA approved VEGF inhibitor pegaptanib, aptamers with a bacteriostatic effect on *Salmonella* have been reported.
Besides the isolation of single specific aptamers for diagnostic or therapeutic aptamers, we focus on the generation of specific aptamer pools, containing aptamers directed against different binding sites to produce affinity materials to specifically capture bacteria from a solution for therapeutic or diagnostic applications.
In contrast to antibodies, aptamers can be selected *in vitro* completely. This is typically done by a systematic evolution of ligands by exponential enrichment (Selex). The Selex process starts with a pool of randomized aptamers, which applied to the target of choice. Aptamers possessing a specificity against the target are able to bind, while unbound aptamers are removed by several washing steps. Bound aptamers are then eluted from the target and amplified by polymerase chain reaction. This generates a new pool of aptamers with the enriched specific aptamers, which are subjected to a new round of selection. After a certain number of rounds resulting aptamers can be analyzed by sequencing to gain single sequences, which can be synthesized and further analyzed. During the process, the enrichment of specific sequences is tracked by introducing fluorescent labels to the aptamers and measuring the signal in the eluted fractions.
Due to their high specificity and chemical stability selected aptamers are then immobilized to generate materials with high affinity towards the bacteria. This would allow to capture a distinct species from a mixture of different bacteria. The introduction of a further phase to such a material, containing antibiotic agents, could then make it possible to extract and dispatch specific bacteria, while other unbound bacteria are not affected.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

318/DVP

Detection of *Bacillus anthracis* spores by Fluorescence in situ Hybridization

R. Jeske*[1], T. Sieper[1], H. von Buttlar[1], K. Aistleitner[1]
[1]*Institut für Mikrobiologie der Bundeswehr, Bakteriologie, München, Germany*

Bacillus anthracis, the causative agent of anthrax, has long been handled as a potential candidate in biological warfare and bioterrorism. Especially since the anthrax attacks of 2001 a resurgence of interest lead to an increasing demand for rapid and sensitive detection methods. This task is hindered by the capability of *B. anthracis* to form highly persistent spores being also the cause for its pathogenic nature. Current state of the art methods to detect *B. anthracis* are either PCR-based or immunological approaches which allow high throughput and short turnaround times. However, these methods can"t discriminate between live and dead cells or contamination with DNA or plasmids.
Fluorescence in situ hybridization (FISH) is an imaging technique based on the detection of ribosomal RNA and can be used for the visual verification of bacteria in different matrices enabling the simultaneous detection of various organisms. Since the detection of an organism via FISH requires at least 400 ribosomal targets per cell only intact and living cells will be stained.
Here we present a step-by-step protocol for the permeabilization of *B. anthracis* spores demanded for FISH. We developed a direct on-slide technique lowering the required amount of spores by several magnitudes compared to present approaches. Accessibility was achieved by targeting the different layers of the exosporium with enzymatic and chemical digestions without lysing the inner core.
PFA-fixed spores of *B. anthracis Sterne and CDC1040* were detected with fluorescent labeled oligo-nucleotide probes addressing bacteria in general (EUB338), low GC-containing bacteria (LGC354) for the detection of Gram-positives and a probe specific to the *Bacillus cereus* group (Bac1157L). Non-complementary DNA probes (nonEUB338) were used as negative controls. This optimized approach enables the visual detection of viable *B. anthracis* spores within 5 hours with a detection rate up to 70% and can be used supplementary to current detection methods.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

319/DVP

***In vivo* biofilm-labelling for analyses of living pathogen populations**

C. Krämer*[1], A. Obermeier[1], R. Burgkart[1]
[1]*Klinikum rechts der Isar, Klinikum für Orthopädie und Sportorthopädie, München, Germany*

The complex mechanisms of biofilm development and population structure of pathogenic bacteria involved in those is still under intensive investigation, although their impact on medical device implementation and patient health by biofilm-associated infection is a known issue [1]. To elucidate the antimicrobial efficiency of modified implant surfaces, the success of biofilm-associated infection prevention measures, and to assess patient-borne biofilm samples, conventional analyses based on plate count tests do not allow further investigation of antimicrobial action on biofilm layers.
Therefore, the development of novel in vivo analyses using fluorescence time-lapse imaging were developed to determine the growth and viability of cell populations in medical relevant pathogenic biofilm forming microorganisms as previously

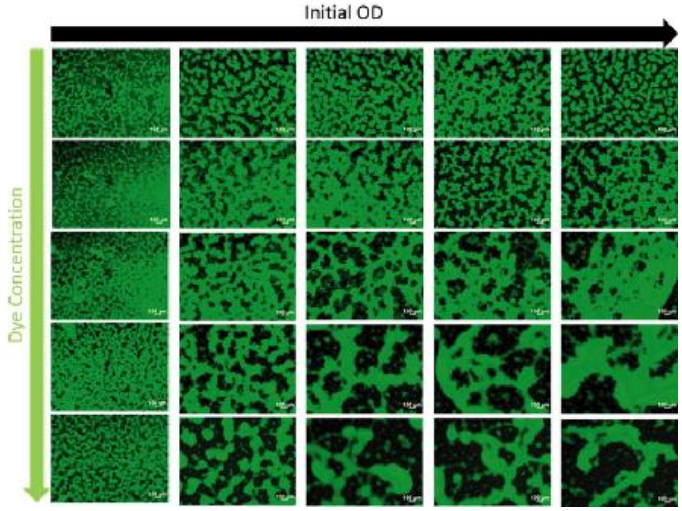
reported for isogenic single-cell wild type analyses [2]-[3]. Our poster presents in vivo fluorescence labelling strategies for relevant implant-associated pathogens and the development of those dynamic staining methods. We proofed the dynamic staining methods were applicable to genetically unmodified wild types of *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *S. epidermidis, Escherichia coli*, and the yeast *Candida albicans.* Our approach comprised the test of commercially available fluorescent dyes for staining of bacteria and yeast. The genetic modification of bacterial biofilm isolates is not required using noninvasive dynamic staining during cultivation. The critical testing of the potential, given by dynamic staining procedures, will be shown for imaged based analyses of antimicrobial medical device surfaces and implants in the future.

Figure 1: Biofilm formation of *E. coli* ATCC 25922 at increasing initial OD600 on a 96 well plate surface after 1 d cultivation with different concentrations of a fluorescent dye. The initial cell number showed to influence the biofilm formation in duration of development, whereas high dye concentrations had impact of the biofilm geometry. Biofilm growth with low dye concentrations in the medium remained unaltered.

References

[1] Ramasamy, M., and Lee, J. Biomed. Res. Int. 2016, 2016: 1851242
[2] Krämer, C., Singh, A., Helfrich, S, Grünberger, A., Wiechert, W, Nöh, K, Kohlheyer, D. PLOS One 2015, 10 (10): e0141768
[3] Krämer, C., Wiechert, W., Kohlheyer, D. Sci. Rep. 2016, 6: 32104

Figure 1



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

320/DVP

The effect of involved *Aspergillus* species on galactomannan in bronchoalveolar lavage of patients with invasive aspergillosis

M. T. Hedayati*[1,2], M. Taghizadeh-Armaki[1,2,3], V. Moqarabzadeh[4], S. Ansari[2], S. M. Omran[3], H. Zarrinfar[5], S. Saber[6], P. Verweij [7], D. Denning[8], S. Seyedmousavi[1,7,9,10]
[1]*Mazandaran University of Medical Sciences, Invasive Fungi Research Center, Sari, Islamic Republic Of Iran*
[2]*Mazandaran University of Medical Sciences, Department of Medical Mycology and Parasitology, School of Medicine, Sari, Islamic Republic Of Iran*
[3]*Babol University of Medical Sciences, Department of Medical Mycology and Parasitology, School of Medicine, Babol, Islamic Republic Of Iran*
[4]*Tehran University of Medical Sciences, Department of Biostatistics, School of Health Sciences, Tehran, Islamic Republic Of Iran*
[5]*Mashhad University of Medical Sciences, Department of Medical Mycology and Parasitology, School of Medicine, Mashhad, Islamic Republic Of Iran*
[6]*Tehran University of Medical Sciences, School of Medicine, Tehran, Islamic Republic Of Iran*
[7]*Radboudumc, Department of Medical Microbiology, Nijmegen, Netherlands*
[8]*University of Manchester, The National Aspergillosis Centre, University Hospital of South Manchester, Manchester, United Kingdom*
[9]*ErasmusMC, Department of Medical Microbiology and Infectious Diseases, Rotterdam, Netherlands*
[10]*National Institutes of Health (NIH), , Laboratory of Clinical Infectious Diseases, National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, Maryland, United States*

Background: The detection of circulating galactomannan (GM) antigen in serum and bronchoalveolar lavage (BAL) fluid is an important surrogate marker for the early diagnosis and therapeutic monitoring of invasive aspergillosis, regardless of the involved species of *Aspergillus*. In the present study we compared the Platelia GM index in BAL of patients with proven and probable pulmonary aspergillosis due to *Aspergillus flavus* versus *A. fumigatus.*
Material and method: In a prospective study between 2009 and 2014, a total of 116 BAL samples were collected from patients with underlying pulmonary disorders referred to two university hospitals in Tehran, Iran. The Platelia *Aspergillus* GM EIA (Bio-Rad Laboratories, Marnes-la-Coquette, France) was used to detect the presence of GM on BAL fluid specimens, according to instruction of manufacturer.
Results: According to EORTC/MSG criteria, 35 patients were classified as IA patients, of which 33 cases had positive GM above 0.5 and 22 cases with GM index ≥ 1. Twenty eight were culture positive for *A. flavus* and seven for *A. fumigatus*. The GM index for *A. flavus* cases was between 0.5-6.5 and those of *A. fumigatus* ranged from 1 to 6.5. The sensitivity and specificity of GM index ≥ 0.5 in cases with *A. flavus* were 89.3% and 100% and those of *A. fumigatus* were 100% and 100%, respectively. The mean of GM index in IA patients with *A. fumigatus* (3.1) was significantly higher than those of *A. flavus* (1.6), (P = 0.031). In the patient group selected for a high likelihood of IA, the sensitivity of GM was lower for *A. flavus* compared to *A. fumigatus.*
Conclusion: This finding might have implications for diagnosis in hospitals and countries with a high proportion of *A. flavus* infections.
Key words: Galactomannan, Bronchoaveolar lavage, *Aspergillus flavus*, *Aspergillus fumigatus*

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

321/DVP

Early and Rapid Detection of Respiratory Pathogens: A Commercial Multiplex PCR Assay in Comparison with Culture

D. Schmidt*[1], J. Buer[1], P. M. Rath[1], J. Steinmann[1]
[1]*University Hospital Essen, Medical Microbiology, Essen, Germany*

Introduction: Hospital acquired pneumonia is a frequent and severe complication in intensive care unit patients with a mortality rate between 25 to 50%. Early and appropriate antibiotic treatment improves prognosis. However, conventional microbiology is time-consuming. At best, culture plus susceptibility results are available 48h after the sample was taken if the pathogen can be cultured at all. The Unyvero Application (Curetis AG, Holzgerlingen) is a semi-automated microbiological analysis system that combines sample preparation and qualitative pathogen detection in a disposable cartridge. The system can currently detect 21 pathogens and 17 antibiotic resistance markers in eight parallel multiplex end-point PCR reactions followed by hybridization onto an array. Time-to-result is below five hours with minimal hands-on-time.
Objectives. The aim of the study was to evaluate the performance of this assay and to assess the agreement between the Unyvero Application and culture.
Materials and methods: Over a period of seven months we have analyzed 146 respiratory specimens of 116 critically ill patients (61% male, 39% female) from the age of 3 months to 85 years (median= 61.5 years).
Results: In general, the results of 142 of 146 cartridges could be evaluated. 52 specimens (36.6%) were positive by the Unyvero Application and 90 specimens (63.4%) were negative. In contrast 32 specimens (22.5%) were positive by culture and 110 specimens (77.5%) were negative. Sensitivity of the assay compared to culture was 91%, the negative predictive value was 97%. Detection rate of the assay was significantly higher than detection rate of culture (79 pathogens by Unyvero vs. 38 pathogens by culture), especially for pathogens that are sensitive to environmental influences (e.g. *Streptococcus pneumoniae*, *Haemophilus influenzae*). As for resistance markers the assay detected four out of five multidrug resistant pathogens in comparison with culture. Additionally, a time-to-result reduction by 75% could be achieved by the assay (assay: median 7.25 hours,culture: median 48 hours).
Conclusion: The Unyvero Application is a useful diagnostic tool for the early detection of pathogens in respiratory specimens from critically ill patients.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

322/DVP

Preparation of new medium from plant materials for isolation and identification of *Acinetobacter* spp.

N. Kazemi Pour*[1], F. Rokhbakhsh Zamin[1], B. A. Chopade[2]
[1]*Islamic Azad University, Kerman Branch, Microbiology, Kerman, Islamic Republic Of Iran*
[2]*Dr.Babasaheb Amberkar Marathwada University, School of life Science, Aurangabad, India*

Backgrounds and aim: The goal of this study was to develop solid media to allow simply screening of clinical and environmental samples for the presence of *Acinetobacter* strains. *Acinetobacter* species are widely distributed in nature as well as in hospital environments. This opportunistic pathogen is highly antibiotic resistant and causes some nosocomial infections. Although there is an increase in its significance and frequency, but it is not a correct approval of their potential importance. It is so, because in routine work there is not a reliable, sensitive, selective and differential medium for isolation of *Acinetobacter*. On the other hand, in research work, scientists must use several different media at the same time to prevent overlooking of these bacteria from samples. In general, a few media are available for isolation of *Acinetobacter* spp. and none of them have a good and reliable differential activity.
Methods: Chopade-Kazemi (CK) VEG medium is the first differential and indicator solid medium used for isolation and identification of *Acinetobacter* strains prepared from plant materials. The plates are easy to prepare. The essential components are 5% (w/v) extract of *Vigna aconitifolia* sprouts, NaCl 5g/l, ox bile 5g/l, crystal violet 0.032 g/l as the indicator and agar 15 g/l. The contribution of each component to the satisfactory function of the plate was studied and defined by using clinical and environmental *Acinetobacter* strains as well as other Gram negative bacteria.
Results: Colonies of *Acinetobacter* could catabolize the test substrate and produce a deep dark blue color, whereas colonies of other Gram negative bacteria fail to show this unique color. In all cases dark blue color of *Acinetobacter* strains was stable from young to old colonies.
Conclusions: CK VEG medium showed more differential activity than the similar media (e.g. Violet red bile agar). New medium was checked by using standard bacteria, different isolates obtained from soil and clinical samples and accordingly, selective and differential activities of CK VEG medium were tested and approved. Finally, it can be concluded that this new medium is useful for easier and faster isolation and identification of *Acinetobacter* spp. from clinical and environmental samples with low cost of preparation.
Key words: Chopade Kazemi (CK) VEG medium, *Acinetobacter* spp., plant components, *Vigna aconitifolia,* sprouts.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

323/DVP

Qualification of rtPCR assays for detection of selected pathogenic microorganisms in a grass-silage biogas process

S. Prowe*[1], K. Kelemen[1], F. Hannemann[1]
[1]*Beuth University of Applied Sciences, Dept 5, Biotechnology, Lab for Microbiology, Berlin, Germany*

Grass and corn silage, dung, green and bio waste are used as substrates for biogas processes. Infected substrate might contaminate the whole biogas plant, including the material circuit including the use of digestate for agricultural use. In addition, biological markers are still missing as parameter for control of biogas processes [1], but were part of the network project BiogasMarker.
A rapid detection and reproducible quantification using qPCR was established in order to evaluate the risk of pathogens during a biogas process. Therefore, during the project Pathogen diagnostic within biogas reactors the development of qPCR based detection systems for some selected relevant pathogens was established.
In addition to available detection systems [2, 3, 4, 5], the focus was especially on phyto- and human pathogenic microorganism which had been selected by a risk priority analysis [6] such as *Xanthomonas translucens, Clostridium difficile*, *C. sordellii and L. monocytogenes.* For these assays, an appropriate plasmid vector system for the quantification was established. An inter-laboratory assay revealed the need of an optimization and validation of all protocols [7]. The associated protocols were validated and the experimental determination of LOD and LOQ were performed. An internal amplification control was also included in all single- and multiplex PCRs. Spiking experiments did check for interferences.
The validation was successfully performed using extraction and quantification of biogas samples from different biogas plants. All established single- and multiplex assays were able to comply with the requirements and might now provide a tool for the identification of key pathogens shown to be most relevant within

biogas plants. This might enable the definition of biological qPCR parameters for further regulations to control safety of biogas processes.

References

[1] DIN 38414 und VDI 4630
[2] Martine Maes, Paolina Garbeva, O. Kamoen (1996), *Phytopathology* 86: 63.
[3] Sabine Nutz, Katharina Döll, Petr Karlovsky (2011), *Analytical and Bioanalytical Chemistry* 401: 717.
[4] Christian D. Ahrberg, Andreas Manz, Pavel Neuzil (2015), *Scientific Reports* 5/11479.
[5] Fröschle, B., Messelhäusser, U., Höller, C., & Lebuhn, M. (2015). Fate of Clostridium botulinumand incidence of pathogenic clostridia in biogas processes. Journal of Applied Microbiology, 119(4), 936.
[6] Alisa Trippner (2013), Master thesis (not published)
[7] Michael Lebuhn*, Jaqueline Derenkó, Antje Rademacher, Susanne Helbig, Bernhard Munk, Alexander Pechtl, Yvonne Stolze, Steffen Prowe, Wolfgang H. Schwarz, Andreas Schlüter, Wolfgang Liebl, Michael Klocke (2016), *Bioengineering* 3, 7.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

324/DVP

Growth limiting effects of biocides to *Listeria monocytogenes*

M. Szendy*[1], A. Rödel[2], S. Al Dahouk[2], R. Dieckmann[2], M. Noll[1]
[1]*Hochschule Coburg, Coburg, Germany*
[2]*Bundesinstitut für Risikobewertung, Fachgruppe Produkthygiene und Desinfektionsstrategien, Berlin, Germany*

Listeria monocytogenes is an important foodborne pathogen and frequently implicated as a causative organism in several outbreaks of foodborne disease. In recent years, an increasing rate of listeriosis has been reported despite the fact that preservative food additives and disinfectants are routinely used in the food industry. However, *L. monocytogenes* can adapt and develop tolerances to biocides and persist over extended periods of time within food products and the food production environment.
About 300 *L. monocytogenes* strains, including field isolates and reference strains of all major serovars (e.g. 1/2a, 1/2b, 1/2c and 4b), were tested under optimal growth conditions (BHI, 24 hours, 37°C) on 96-well microtiter plates in two-fold dilutions for their susceptibility to food additives sodium nitrite (NaNO2; 0.06 – 8mg/ml) and citral (0.6 – 71.2mg/ml). In addition, four compounds commonly used as disinfectants were tested, namely hydrogen peroxide (H2O2; 0.005 – 625mg/ml), sodium hypochlorite (NaOCl; 0.1 – 16mg/ml), quaternary ammonium compound benzalkonium chloride (BAC; 0.0001 – 0.016mg/ml) and cetalkonium chloride (CKC; 0.0003 – 0.016mg/ml). The majority of strains displayed MIC values of 8mg/ml (NaNO2), 8.9mg/ml (Citral), 0.156mg/ml (H2O2), 4mg/ml (NaOCl), 0.004mg/ml (BAC) and 0.001mg/ml (CKC). Generally, the percentages of deviations in MIC values were rather low for the tested strains.
Sub-lethal concentrations of disinfectants or preservatives might contribute to emergence of persistent *L. monocytogenes* strains in food processing facilities with altered biocide and/or antibiotic susceptibilities.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

325/DVP

New strategies for differential diagnosis of bacterial dermatoses via lateral flow

L. Kroschwald*[1], A. Wiedenfeld[1], E. Broese[1], C. Dittrich[1], T. Loewe[1], C. Eichhorn[1], F. Gunzer[1], R. Ahrendt[1], C. Weber[1], A. Bauer[1]
[1]*Universitätsklinikum Carl Gustav Carus, Klinik und Poliklinik für Dermatologie, Dresden, Germany*

Bacterial infections of the skin are the most common diseases with increasing prevalence and a high number of unreported cases. An accurate and rapid detection of pathogenic agents is most important for the choice of an adequate therapy and the success of treatment. Currently, conventional diagnostic is commonly based on microbiological methods such as cultivation, followed by morphological and biochemical identification microscopically, macroscopically or by using automated platforms. Furthermore, antibiotic susceptibility testing is often necessary. This procedure requires long-term experience and is time consuming with cultivation periods of up to several days. Moreover, despite the fact that culture is the gold standard in microbial diagnostic, in comparison to molecular methods culture shows a lower diagnostic sensitivity.
To provide a precise identification of pathogens relevant for skin infections, the aim of this study is the development and clinical validation of a simple, sensitive and effective technique for identification of the most common bacteria directly from a skin swab, offering a new approach for clinical analysis. Based on detection of individual DNA sequences of respective pathogens, this assay enables reliable results within hours.
By combining a new generation of multiplex-polymerase chain reaction (PCR) and hybridization of the amplified DNA with pathogen-specific DNA-probes and subsequent detection on test strips, the system fulfills extended quality standards while maintaining already achieved performance. At the same time we pursued improvement of further test parameters such as user-optimized handling, time saving and species specific recognition.
The validated test-kit will allow a timely recognition of causative bacteria in acute and chronic skin infektions, helping to prevent re-infections and to assure a rapid and targeted therapy. The benefit for the patient is an improved treatment success, and also a reduced duration of treatment or avoidance of unnecessary treatment. Furthermore, this cost-efficient platform takes account of the requirements of remuneration regulations in the health care sector.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

326/DVP

Virus detection by use of a novel label-free biosensor technology

D. Vollandt*[1], G. Proll[2], F. Pröll[2], M. Niedrig[1]
[1]*Robert Koch Institute, Center for Biological Threats and Special Pathogens 1, Highly Pathogenic Viruses, Berlin, Germany*
[2]*Biametrics GmbH, Tübingen, Germany*

Fast and early diagnoses of viral infections are essential for an efficient treatment of the patient and a timely outbreak management. Current detection strategies for pathogens like Ebola, Zika and Yellow fever virus are based on cost- and time-consuming multistep methods and can only be performed by specially trained personnel.
The aim of this project is to develop and validate a biosensor-based multiplex immunoassay for the rapid and easy detection of viruses as well as virus-specific antibodies from complex biological samples. A parallel spotting of different catcher molecules on a biosensor allows the simultaneous detection of several targets. The innovative SCORE technology (Single Colour Reflectometry) enables a time-resolved and label-free detection of target molecules.

After developing an optimal coating procedure of the biosensor with virus-specific antibodies, different clinical relevant Flaviviruses (Tick-borne encephalitis virus, Yellow-fewer virus, Dengue virus) have been tested concerning their detection under different assay conditions. Moreover, the temperature- and buffer-dependency of the antibody-virus-interactions were analyzed. The optimization steps resulted in a high reproducibility of the measurements. The sample-to-result-time takes less than one hour, the measurement itself less than 15 minutes. In further experiments the specificity and sensitivity of this method will be compared to conventional procedures like IFA and ELISA.
This innovative method is rapid, reliable, has low current costs and is easy to handle.
These characteristics enable also an application in the field as a point-of-need diagnostic. Beside the direct detection of viruses or virus-specific antibodies, this biosensor technology has a high potential for many other fields of diagnostic or biology, e.g. the detection of bacterial pathogens, kinetic studies or drug discovery.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

327/DVP

Development of a high-resolution melting analysis assay for rapid and high-throughput identification of clinically important dermatophyte species

T. Shokohi*[1], M. Didehdar[2], B. Khansarinejad[3], N. Amirrajab[4]
[1]University of Medical Sciences, Department of Medical Mycology and Parasitology, Invasive Fungi Research Center (IFRC), School of Medicine, Sari, Islamic Republic Of Iran
[2]University of Medical Sciences, Department of Medical Mycology and Parasitology, Invasive Fungi Research Center (IFRC), School of Medicine, Sari, Islamic Republic Of Iran
Arak University of Medical Sciences, Department of Medical Mycology and Parasitology, Arak, Islamic Republic Of Iran
[3]Arak University of Medical Sciences, Molecular and Medicine Research Center, Arak, Islamic Republic Of Iran
[4]Mazandaran University of Medical Sciences, Student Research Committee, Sari, Islamic Republic Of Iran

Background: Accurate identification of dermatophyte species is important both for epidemiological studies and for implementing antifungal treatment strategies. Although nucleic acid amplification-based assays have several advantages over conventional mycological methods, a major disadvantage is their high cost.
Objective: The aim of this study was to develop a rapid and accurate real-time PCR-based high-resolution melting (HRM) assay for differentiation of the most common dermatophyte species.
Materials and Methods: The oligonucleotide primers were designed to amplify highly conserved regions of the dermatophyte ribosomal DNA. Analysis of a panel containing potentially interfering fungi demonstrated no cross reactivity with the assay.
Results: To evaluate the performance characteristics of the method, a total of 250 clinical isolates were tested in comparison with the long-established PCR-RFLP method and the results were reassessed using DNA sequencing, as the reference standard method. The assay is able to type dermatophytes using normalised melting peak, difference plot analysis or electrophoresis on agarose gel methods. The results showed that, in comparison to PCR-RFLP, the developed HRM assay was able to differentiate at least 10 common dermatophytes species with a higher speed, throughput and accuracy.
Conclusion: These results indicate that the HRM assay will be a useful sensitive, high throughput and cost-effective method for differentiating the most common dermatophyte species.
Key words: Dermatophyte, identification, HRM analysis

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Environmental Microbiology and Ecology (FG EE)

328/EEP

Biosynthesis of methylmenaquinone

S. Hein*[1], O. Klimmek[1], J. Simon[1]
[1]TU Darmstadt , Microbial Energy Conversion and Biotechnology, Darmstadt, Germany

Introduction: The membranous quinone/quinol pool is essential for the majority of life forms and has been widely used as an important biomarker in microbial taxonomy. In the anaerobic world, the most important quinones are menaquinone (MK) and a methylated form of MK, designated methylmenaquinone (MMK), which is considered to serve specifically in low-potential electron transport chains involved in anaerobic respiration.
Objectives: This work aimed to understand the enzymology of bacterial MMK production in the context of the two known menaquinone biosynthesis pathways, i.e. the classical Men pathway and the more recently discovered Mqn or futalosine pathway.
Materials & methods: The MMK-producing Epsilonproteo-bacterium *Wolinella succinogenes* was used as a model organism. Quinones were extracted from membranes, separated by HPLC and analysed by UV absorption spectroscopy and mass spectrometry. Genetically engineered *W. succinogenes* mutants were constructed and heterologous production of MK methyltransferases in *W. succinogenes* or *Escherichia coli* was achieved. An *in vitro* MK methylation assay was established.
Results: A phylogenetically widespread class C radical *S*-adenosylmethionine methyltransferase (RSMT) was identified to catalyse MMK production using MK as substrate. Such enzymes, termed either MenK or MqnK, are present in MMK-producing bacteria (and some archaea) that possess either the Men or the Mqn pathway. A *W. succinogenes mqnK* deletion mutant was unable to produce MMK but its formation was restored upon genomic complementation using either the native *mqnK* gene or *menK* from *Adlercreutzia equolifaciens*, a human gut bacterium, or *Shewanella oneidensis*. Moreover, expression of *A. equolifaciens menK* enabled *E. coli* cells to produce MMK and a methylated form of 2-demethylmenaquinone.
Conclusion: The elucidation of MMK biosynthesis is an important finding in the context of microbial anaerobic physiology and demonstrates an unprecedented function for a class C RSMT enzyme in primary cell metabolism. It also offers the prospect to design artificial methylated quinones *in vivo*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

329/EEP

Growing on laughing gas as sole electron acceptor: the case of *Wolinella succinogenes*

S. Hein*[1], S. Witt[1], J. Simon[1]
[1]TU Darmstadt , Microbial Energy Conversion and Biotechnology, Darmstadt, Germany

Introduction: Nitrous oxide (N_2O; laughing gas) is a powerful greenhouse gas and a major cause of ozone layer depletion. During the last century, human activities severely increased N_2O emissions, mainly from agriculture, livestock farming and wastewater treatment. Numerous pathways for biological N_2O production have been described including nitrate/nitrite reduction via nitric oxide and N_2O to N_2 (denitrification) as well as dissimilatory nitrate/nitrite reduction to ammonia (DNRA). Only

one type of enzyme has been found to be involved in biological N_2O consumption and this copper-dependent N_2O reductase (NosZ) is employed in denitrifiers and DNRA organisms. Recently, however, two distinct phylogenetic groups of NosZ enzymes and their *nos* gene clusters named clade I and clade II N_2O reductase (systems) have been identified. The environmental impact of organisms using clade II NosZ enzymes is currently unresolved but potentially significant.

Objectives: The main aim of this study was to elucidate the architecture of the electron transport chain that enables anaerobic N_2O respiration in the nitrate-ammonifying model Epsilonproteobacterium *Wolinella succinogenes*, which harbours a clade II cytochrome *c* N_2O reductase and an atypical *nosZ*, *-B*, *-D*, *-G*, *C1*, *-C2*, *-H*, *-F*, *-Y*, *-L*, gene cluster.

Materials & methods: Various genetically engineered *nos* gene cluster mutants of *W. succinogenes* were constructed and their ability to grow by N_2O respiration with formate as electron donor was examined. The cellular content of NosZ was estimated by haem staining.

Results: *W. succinogenes* cells, originally isolated from bovine rumen fluid, were shown to grow by N_2O respiration with a doubling time of about 1.1 h. The corresponding growth yield per mole formate significantly exceeded those of fumarate and nitrate respiration. Based on the phenotypes of appropriate non-polar gene deletion mutants, the potential electron transfer proteins NosG, NosH, NosC1, NosC2 and NosB were shown to be indispensable for N_2O respiration. Unexpectedly, the *qcrB* and *-C* genes encoding the haem-containing subunits of the cytochrome bc_1 complex were also found essential.

Conclusion: We present a revised model of the proton motive electron transport chain catalyzing *W. succinogenes* N_2O respiration, which is prototypical for clade II organisms. Our findings challenge the previously proposed menaquinol-oxidising function of the NosGH complex and of homologous proteins involved in other forms of anaerobic respiration.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

330/EEP

Investigation of the different routes of nitrogen reduction in denitrification beds as a function of carbon source and microbiome

V. Grießmeier*[1], A. Bremges[2,3], A. McHardy[3], J. Gescher[1]
[1]*Karlsruhe Institute of Technology (KIT), Department of Applied Biology, Karlsruhe, Germany*
[2]*Helmholtz Centre for Infection Research, Computational Biology of Infection Research, Braunschweig, Germany*
[3]*German Center for Infection Research (DZIF), Braunschweig, Germany*

Field denitrification beds that contain polymeric plant material are increasingly used to eliminate nitrate from agricultural drainage waters. Still, knowledge regarding the microbial composition, potential synergistic effects, and the overall carbon degradation process of these systems is sparse. This study indicates that while migrating through drainages, agricultural waste waters are metabolized differently under warm and cold conditions. While fertilizers contained ammonium and nitrate, ammonium was the main nitrogen compound in the drainage waters during the warmer summer months. This is a challenge for these bed systems since they are constructed as bioreactors for the denitrification, rather than the nitrification, of nitrogen compounds. Interestingly, wood chips as a carbon source of denitrification reactors sustained the development of an archaeal community consisting of methanogenic species and members of the Thaumarchaeota phylum, while archaea were not present in bioreactors operated with wood pellets. Wood chips select for the development of a diverse microbial community that is especially suited to the removal of low nitrate concentrations. Under these conditions there seems to be a division of labor, with Clostridia inhabiting the wood chip surface and degrading the polymeric material, and Proteobacteria—primarily localized in the planktonic phase—most likely using fermentation end products as electron donors for denitrification. Interestingly, higher nitrate concentrations can lead to an activity switch from denitrification to dissimilatory reduction of nitrate to ammonium (DNRA). This switch was not significantly observed in reactors operated with wood pellets, in which a stable denitrification process occurred even at high nitrate loading rates.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

331/EEP

Identification of the product of the 5,6,7,8-tetrahydro-2-naphthoyl-CoA reductase

P. Weyrauch*[1], I. Heker[1], R. U. Meckenstock[1]
[1]*Universität Duisburg-Essen, Biofilm Centre - Aquatic Microbiology, Essen, Germany*

Background: Dearomatising ring-reductases play an important role in anaerobic degradation pathways of aromatic compounds. Naphthalene degradation by sulphate-reducing bacteria is known to proceed via 5,6,7,8-tetrahydro-2-naphthoyl-CoA (THNCoA), which gets reduced to a hexahydro-2-naphthoyl-CoA (HHNCoA) with unknown conformation by an enzyme similar to class I benzoyl-CoA reductases (Eberlein *et al.*, 2013). The downstream pathway was proposed to proceed through β-oxidation-like reactions as indicated by metabolites identified in culture extracts by GC-MS analysis (Annweiler *et al.*, 2002).

Objectives: In previous studies, the respective reaction was measured with NADH as electron donor, but neither could a complete conversion of the added THNCoA be achieved, nor could further metabolites be detected via HPLC analysis. The objectives of our study were to optimise the reductase assay in order to detect further metabolites and to verify that HHNCoA is indeed the final reductase product.

Methods: THNCoA reductase assays were performed with cell free extracts of the sulphate reducing naphthalene degraders N47 and NaphS2 as described previously. Different electron donors and other co-factors were tested and samples were analysed via LC-MS in single ion mode, scanning for expected metabolites.

Results: The more sensitive LC-MS analysis enabled us to detect that small amounts of the product of an HHNCoA hydratase are formed in the standard THNCoA reductase assays with NADH as electron donor. However, the further conversion of this intermediate by an NAD+-dependant β-hydroxyacyl-CoA dehydrogenase was inhibited by the excess of NADH present in these assays. Experiments with alternative electron donors indicated that 2-oxoglutarate is the natural electron donor of the THNCoA reducing system and low-potential electrons are delivered in the form of reduced ferredoxin via a 2-oxoglutarate:ferredoxin oxidoreductase. With 2-oxoglutarate as electron donor, a complete conversion of THNCoA was observed and further downstream metabolites could be detected.

Conclusion: These metabolites correspond to the ones identified in earlier metabolite analyses and indicate a β-oxidation-like downstream pathway with water addition to HHNCoA and a first ring-fission via a hydrolase acting on a β"-hydroxy-β-oxodecahydro-2-naphthoyl-CoA intermediate.

References

[1] Annweiler, E., Michaelis, W., and Meckenstock, R.U. (2002) Identical ring cleavage products during anaerobic degradation of naphthalene, 2-methylnaphthalene, and tetralin indicate a new metabolic pathway. *Appl Env Microbiol* 68: 852-858.
[2] Eberlein, C., Johannes, J., Mouttaki, H., Sadeghi, M., Golding, B.T., Boll, M., and Meckenstock, R.U. (2013) ATP-dependent/-

independent enzymatic ring reductions involved in the anaerobic catabolism of naphthalene. *Env Microbiol* 15: 1832-1841.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

332/EEP
Quantification of *Macrococcus caseolyticus* in bioaerosols by Droplet Digital PCR
A. Grünberg*[1], U. Jäckel[1]
[1]*Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Berlin, Germany*

Introduction and Objectives: In the last decades, livestock farming was increasingly intensified. In this context the risk in the exposure to airborne microorganisms as a part of livestock associated bioaerosols gets more important for employers as well as residents neighbouring these livestock farms. In poultry farms often members of the family Staphylococcaceae dominate the airborne bacterial population [1]. *Macrococcus caseolyticus* has been detected as one of them by cultivation dependent methods and cloning analyses. *M. caseolyticus* is known to be an animal pathogen [2] and is speculated as a possible origin of the methicillin resistant genes found in MRSA [3]. To enable an adequate exposure assessment to this species, in this study a species specific PCR-based detection system should be established and employed for its quantification in bioaerosols from chicken poultry farm.
Material and Methods: Basis for the detection of *M. caseolyticus* in bioaerosols was a species specific target segment within the 16S rRNA gen. First *in silico* analyses using currently available RDP and NCBI sequences were performed. Afterwards "no-template" control strains on species of the same genus (with only minor sequence differences) as well as on species of other genera in the family Staphylococcaceae were chosen and employed to adjust the highest process specificity in a conventional PCR-system. The specificity was investigated by PCR product analysis due to the generation and of two obtained clone libraries from a turkey poultry house. Furthermore 30 bacterial isolates obtained from bioaerosol samples and harbouring 5 *M. caseolyticus* strains were investigated for sensitivity analyses of this PCR system. After adaption of PCR conditions to a Droplet Digital PCR system, the new PCR system was used to quantify *M. caseolyticus* in DNA extracts from bioaerosol samples of two chicken poultry farms.
Results and Conclusion: All 5 *M. caseolyticus* strains could be detected safely by the new primer system. Furthermore it was shown that all gene sequences, which were obtained from spiked bioaerosols out of a turkey farm and were amplified with the new primer system, could be assigned to the target organism. Based on adjusted PCR conditions for the first time the Droplet Digital PCR was successfully used for the quantification of *M. caseolyticus* in bioaerosol samples. It could be shown that *M. caseolyticus* are ubiquitary present in numbers of up to 10^3 cells per m^3 of air in the chicken poultry houses.

References
[1] Schneider, D. et al. (2015). "Taxonomische Charakterisierung luftgetragener Bakterien der Familie Staphylococcaceae in Emissionen von Hähnchenmastanlagen (Bedeutung für die Gesundheitsbeurteilung)." Gefahrstoffe - Reinhaltung der Luft 75(9): 340-346.
[2] TRBA 466. "Einstufung von Prokaryonten (Bacteria und Archaea) in Risikogruppen). "
[3] Baba, T. et al. (2009). "Complete genome sequence of *Macrococcus caseolyticus* strain JCSCS5402, reflecting the ancestral genome of the human-pathogenic staphylococci". J Bacteriol 191(4): 1180-90.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

333/EEP
Detection and Quantification of Sulfate-Reducing and Polyaromatic Hydrocarbons-Degrading Bacteria using Real-Time Quantitative PCR
W. Ismail*[1], B. Nasser[2], M. Mohamed[2], R. Hamzah[1]
[1]*Arabian Gulf University, Life Sciences, Mananma, Bahrain*
[2]*Saudi Aramco, Research and Development Center, Dhahran, Saudi Arabia*

Question: Although microbial biotechnology can be potentially applied throughout the value chain of the petroleum industry, it has not been exploited to its full potential. One reason for this is the lack of sufficient understanding of the diversity and ecophysiology of the involved microbes. Culture-dependent microbial characterization techniques are not sufficiently efficient and are time consuming. To the contrary, culture-independent molecular techniques are fast and efficient in detecting and quantifying microorganisms in environmental samples.
Methods: In this study, quantitative real-time polymerase chain reaction (RT-qPCR) was adopted to detect and quantify two groups of microbes known to play a significant role in petroleum recovery and processing. These are the sulfate-reducing bacteria (SRB) and polycyclic aromatic hydrocarbons (PAH)-degrading bacteria. Water samples were collected from an oilfield bypass (Qutaif junction, Saudi Arabia) pipeline system. In addition, soil and sludge samples were collected from Abo Ali plant (injection water treatment plant) and Khafji GOSP (Gas-Oil Separation Plant, Saudi Arabia). SRB were detected by targeting a fragment of the *apsA* gene encoding adenosine-5-phosphosulfate reductase, which is characteristic of all SRB. The PAH-degrading bacteria were detected using a primer pair that amplifies a fragment of the gene encoding the large subunit of the naphthalene dioxygenase gene.
Results: The naphthalene dioxygenase gene was detected in almost half of the soil samples. The highest copy number was detected in soil sample number 11 with 60540 copies/g soil. Most of the analyzed water samples contained high copy numbers of the naphthalene dioxygenase gene. The highest copy number was detected in sample 15 with 3846 copies/ml. In general, the copy numbers in the soil samples were higher than those detected in the water samples. Most of the analyzed water samples revealed the presence of high copy numbers of the *apsA* gene. The highest copy number was detected in water sample number 2. Only 7 of the soil samples revealed the presence of the *apsA* gene. The highest copy number was detected in soil sample number 11.
Conclusions: RT-qPCR is an efficient and fast tool that enables the detection and enumeration of bacteria in oilfields. SRB and PAH-degrading bacteria exist in some Saudi oilfields.

References
[1] Beech, IB., and Sunner, JA. (2007) Sulphate-reducing bacteria and their role in corrosion of ferrous materials. In: .Barton, L-L., and Hamilton, W-A. (eds), Sulphate-Reducing Bacteria. Environmental and Engineered systems. Cambridge University Press, Cambridge, pp. 459-482.
[2] Ben-Dov, E., Brenner, A., and Kushmaro, A. (2007) Quantification of sulfate-reducing bacteria in industrial Wastewater by Real-time Polymerase Chain Reaction (PCR) Using *dsrA* and *apsA* Genes. Microbial Ecology 54 (3): 439-51.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

334/EEP

Chasing microorganisms involved in cryptic sulfate reduction in sediments of a large pre-alpine lake, Lake Constance

J. Thiel*[1,2], M. Pester[1]
[1]*University of Konstanz, Biology, Konstanz, Germany*
[2]*University of Konstanz, Konstanz Research School Chemical Biology, Konstanz, Germany*

Microbial sulfate reduction in aquatic sediments is a process of high relevance for the global carbon and sulfur cycle. In contrast to marine sediments, sulfate concentrations in freshwater sediments are very low. It was therefore generally assumed that sulfate reduction in freshwater environments has a minor role for element cycling. However, this is contradicted by the high sulfate reduction rates (SRR) regularly observed in lake sediments and other freshwater habitats. To explain such high SRR despite the prevailing low sulfate concentrations, a cryptic sulfur cycle was proposed to occur in freshwater habitats (Pester, Knorr et al. 2012). Lake Constance is a large oligotrophic lake, where high sulfate reduction rates of up to 2000 nmol L^{-1} day^{-1} have been observed (Bak and Pfennig 1991). Building upon this observation, we setup anoxic sediment microcosms in the presence and absence of small periodic sulfate amendments (200 µM). Individual microcosms were incubated in the presence of one of the following typical organic matter degradation intermediates (ca. 200 µM): formate, acetate, lactate, propionate, butyrate, and no-substrate control. To discern the effect of sulfate amendment on the degradation of individual organic substrates, we followed the production of methane and carbon dioxide as well as the turnover of sulfate, and short-chained fatty acids/lactate. Methanogenesis was reduced by 18−58% in sulfate-amended microcosms while there was no effect on carbon dioxide production and substrate degradation products. Substrate amendment had no major effect on mineralization, indicating high internal substrate concentrations. Currently, we follow changes in bacterial and archaeal 16S rRNA and 16S rRNA genes in the individual setups using next generation amplicon sequencing. The combination of these microbial community responses and the obtained activity profiles will help us to identify microorganisms actively involved in cryptic sulfate reduction in Lake Constance sediments.

References

[1] Bak, F. and N. Pfennig (1991). Fems Microbiology Ecology 85(1): 31-42.
[2] Pester, M., K. H. Knorr, M. W. Friedrich, M. Wagner and A. Loy (2012). Frontiers in Microbiology 3.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

335/EEP

MccA-dependent sulfite respiration in *Wolinella succinogenes*: the role of MccC, MccD and MccL

J. Eller*[1], J. Simon[1]
[1]*Technische Universität Darmstadt, Department of Biology, Darmstadt, Germany*

Introduction: The sulfite anion (SO_3^{2-}) causes damage to proteins, nucleic acids and lipids and is used as disinfectant, antioxidant and food preservative. Nonetheless, the Epsilonproteobacterium *Wolinella succinogenes* uses sulfite as terminal electron acceptor in anaerobic respiration. The novel copper-containing octahaem cytochrome *c* MccA serves as periplasmic sulfide-producing sulfite reductase. MccA is encoded by the first gene of the *mcc* gene cluster, which is thought to be regulated by the MccRS two-component system. Conceivably, the periplasmic iron-sulfur protein MccC and the putative quinol dehydrogenase MccD are thought to be involved in electron transport to MccA.

Objectives: Experiments were performed to elucidate the role of MccC, MccD, copper ions and additionally the putative copper chaperone MccL in *W. succinogenes* sulfite respiration.
Materials & methods: Mutants of *W. succinogenes* were constructed that lacked *mccC*, *mccD* or *mccL*. Their ability to grow by sulfite respiration with formate as electron donor was examined under copper-sufficient or copper-limited conditions. The cellular content of MccA was monitored by haem staining. In contrast to previous experiments, all mutants possessed a frameshift-corrected *mccR* gene.
Results: The presence of an intact *mccR* gene resulted in rapid MccA synthesis after the addition of sulfite to fumarate-grown cells. Mutants *mccC* or *mccD* were severly hampered in growth by sulfite respiration and sulfite turnover despite the presence of mature MccA. Copper depletion led to significantly reduced sulfite conversion rates, a phenotype similar to that of cells lacking the *mccL* gene.
Conclusion: The results indicate that MccC and MccD are obligatory components of the electron transport chain that drives sulfite respiration in *W. succinogenes*. Furthermore, it is suggested that the maturation of the haem *c*:copper active site of MccA is facilitated by the copper chaperone MccL.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

336/EEP

Isolation and characterization of some bacterial strains inhibiting several human skin pathogens

H. G. Song*[1], D. S. Lee[1]
[1]*Kangwon National University, Biological Sciences, Chuncheon, South Korea*

To date chemical preservatives, such as parabenes have been widely used in food and cosmetic industries, but due to their toxicity and other side-effects they should be replace to safe ones. Recently there have been numerous studies on development of effective and unharmful antiseptics from natural substances of plants and microorganisms. This study was carried out to evaluate effects of antimicrobial substances produced by isolated bacteria from various soils of South Korea. Among several hundreds of bacterial strains isolated, *Paenibacillus elgii* DS381 (from Chuncheon City), *Bacillus subtilis* DS660 (from Yeongwol City) and *Paenibacillus peoriae* DS842 (from Daekeum Cave) showed high antimicrobial activities against human skin pathogens such as *Staphylococcus aureus*, *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus niger*. The mechanisms of antimicrobial activity of these bacteria were also examined. Isolated strains inhibited growth of all human skin pathogens tested. DS381, DS660 and DS842 showed 400, 800 and 200 AU/ml of maximum antimicrobial activity. When antimicrobial mechanisms were examined, strains DS660 and DS842 produced 0.056±0.009 and 0.17±0.015 mmol/ml of siderophore, respectively, and strain DS381 showed 1.56±0.13 U/ml of chitinase activity. Strains DS660 and DS842 also showed 167.29±9.98 and 357.28±13.73 nmol/min/mg of β-1,3 glucanase activity, respectively. In addition, oil spreading test and thin layer chromatography of ethyl acetate extract of strains DS381, DS660 and DS842 culture supernatant suggested production of biosurfactants such as lipopeptide and glycolipid. Culture supernatants of strains DS381, DS660 and DS842 were able to reduce the surface tension to 41, 33.5 and 33.5 mN/m. Antimicrobial substance of all three strains were stable at broad range of temperature (-22~121℃), pH (3~12) and chemical compounds (urea, Triton X-100, Tween 20 and Tween 80). These results suggest that *Paenibacillus elgii* DS381, *Bacillus subtilis* DS660 and *Paenibacillus peoriae* DS842 may be utilized as an environment-friendly biocontrol agent against some important human skin pathogens for commercial products such as cosmetics.

Figure 1

Table 1. Minimum inhibitory concentration (MIC) determined of strains DS381, DS660 and DS842 by broth microdilution method.

Target organism	DS 381 (mg/ml)	DS 660 (mg/ml)	DS 842 (mg/ml)
C. albicans	2.12	5<	5<
B. subtilis	0.39	1.25	1.25
S. aureus	0.78	5<	5<
A. niger	1.56	5<	5<
P. aeruginosa	0.39	1.25	5<
E. coli	0.78	5<	5

Figure 2

Table 2. Inhibition of target organism by culture supernatant of strains DS381, DS660 and DS842 as determined by the agar well diffusion test.

Target organism	DS381		DS660		DS842	
	I.D.(mm)	Activity	I.D.(mm)	Activity	I.D.(mm)	Activity
C. albicans	21.0±0.5	+++	17±2.0	++	20±0	++
B. subtilis	24.3±1.1	+++	15.3±2.5	+	18.6±0.5	++
S. aureus	24.6±4.1	+++	26.8±2.5	++++	27.5±1.2	++++
A. niger	23.6±0.5	+++	23.3±1.5	+++	27.3±3	++++
P. aeruginosa	16.3±0.5	++	25.3±1.1	+++	12.6±1.5	+
E. coli	20±0	++	17.6±0.5	++	21±0	+++

I.D. : inhibition zone diameter
*No inhibition zone : -, 11~15 mm : +, 16~20 mm : ++, 21~25 mm : +++, 26 mm~ : ++++

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

337/EEP

Degradation and production inhibition of aflatoxin B_1 by some bacterial strains isolated using Coumarin medium

H. G. Song*[1], H. E. Lee[1]
[1]*Kangwon National University, Biological Sciences, Chuncheon, South Korea*

Various mycotoxins are produced by many fungi and aflatoxin B1 (AFB1) produced mainly by *Aspergillus flavus* is the most well-known and can be found from contaminated cereals. It is extremely toxic and carcinogenic, and poses a severe threat to animal health and brings about huge economic losses. Therefore, production of AFB1 by *A. flavus* has to be inhibited and AFB1 already produced should be removed to prevent economic losses from contamination of crops. This study investigated AFB1 biodegradation ability of some isolated bacteria. Over 100 bacterial strains were isolated from various sources including animal feces. A medium used for bacterial screening contained coumarin as a sole carbon sources. Some of isolated strains showed more than 70% reduction of AFB1 in liquid culture during 72 h incubation. Among them, AF34 strain degraded 72.70±2.89% and 88.44±0.08% of 0.1 and 1.25 mg/L of AFB1, respectively at 30℃. At 37℃, AF34 strain reduced 98.03±0.83% and 90.89±0.46% of 0.1 and 1.25 mg/L of AFB1, respectively after 72 h of incubation. In the above conditions, strains AF38 and AF40 showed 98.08±0.29% and 83.57±0.18% of AFB1 degradation, respectively. Then, inhibition of AFB1 production by *A. flavus* (KACC 44986) was examined in yeast extract sucrose medium with simultaneous inoculation of isolated strains or addition of supernatant of bacterial culture. The control fungal culture showed 11.91±1.69 mg/L of AFB1 production, but the fungal culture with the addition of culture supernatant of AF34, AF38 and AF40 formed 9.76±1.22 mg/L, 10.42±0.34 mg/L and 7.37±3.52 mg/L of AFB1, respectively after 120 h of incubation. Even co-inoculation of isolated bacteria reduced them down to 6.32±5.81 mg/L, 7.59±4.30 mg/L and 0.39±0.50 mg/L, respectively. These results suggest these bacteria can be utilized to inhibit and remove of AFB1 production from valuable resources including various crops.

Figure 1

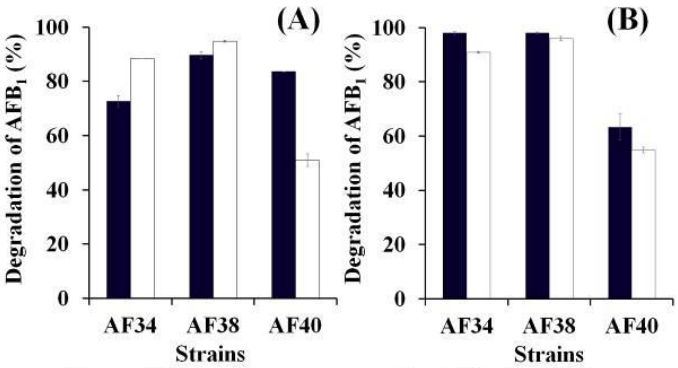


Fig. 1. Effect of temperature (A: 30℃, B: 37℃) on degradation of AFB_1 (■: 0.1 mg/L, □: 1.25 mg/L).

Figure 2

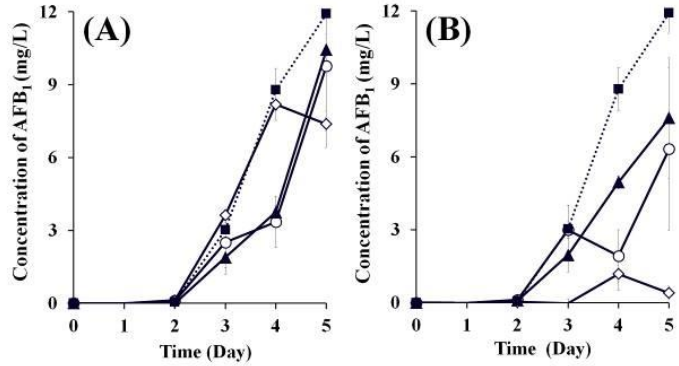


Fig. 2. Inhibition of AFB_1 formation by *A. flavus* with addition of supernatant of bacterial culture (A) and simultaneous inoculation of isolated strains (B). Symbols (■: control, ○: AF34, ▲: AF38, ◇: AF40).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

338/EEP

Investigation of recovery efficiencies in bioaerosol analyses with filtration

I. Schulze-Hessing*[1], U. Jäckel[1]
[1]*Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Berlin, Germany*

Introduction and Objectives: The impact of bioaerosols on ecosystem dynamics and human health, especially at workplaces in animal husbandry, is becoming increasingly evident [1, 2]. Therefore, the microorganism concentration and the community structures in bioaerosols in the environment as well as on work places were intensively studied using different sampling methods (filtration, impingement and impaction). Furthermore various approaches for further analyses like culture-based methods, total cell counting (TCC) and molecular approaches were used. However studies determining the efficiency of bioaerosol sampling and sample processing methods are rare. Thus, the aim of this study was to investigate recovery efficiencies in bioaerosol analyses with filtration.

Materials and Methods: We determined the recovery of different cell species after sampling by filtration with either polycarbonate, quartz fibre or glass fibre filters. A fixed number of cells was spiked on filters, the sampling process was simulated and the recovery efficiency was investigated via TCC with the fluorescent DNA stain 4',6-diamidino-2-phenylindole or DNA extraction and

quantification of the DNA amount by fluorometric measurements. For these analyses four different bacteria species, namely *B. subtilis* subsp. *subtilis*, *M. luteus, P. nitroreducens* and *S. capitis* subsp. *capitis* as well as the yeast *S. cerevisiae* were used.
Results and Conclusion: Using polycarbonate filters the recovery was usually higher than with quartz fibre or glass fibre filters, which have a much coarser surface. Moreover the recovery was generally higher after TCC compared to the molecular approach with DNA-Extraction, probably due to additional effects concerning the recovery during DNA extraction. Nevertheless the differences between the recoveries of various cell species were much more significant. *B. subtilis* and *S. cerevisae* showed much higher recovery efficiencies than *M. luteus, S. capitis* and *P. nitroreducens* e.g. after filtration with glass fibre filters and TCC the recoveries varied between 76 % (*S. cerevisae*) and 1,9 % (*S. capitis*). If these results are transferable one-to-one to real bioaerosol analyses, problems in community analyses by concealing the real composition can be assumed. So the results of this study clearly show that efficiencies of bioaerosol sampling and analyses methods have to be specified to verify the significance of an investigation.

References
[1] Martin, E. *et al.* (2013*) Environ. Sci.: Processes Impacts* 15: 478-484.
[2] Bowers, R. M. *et al.* (2009) *Appl. Environ. Microbiol.* 75: 5121-5130.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

339/EEP
Marine bacterial communities colonizing microplastic surfaces differ from those colonizing natural particles
A. Franco*[1], J. Reichert[2], M. Hardt[3], A. Arnold[2], P. Schubert[2], P. Kämpfer[1], T. Wilke[2], S. Glaeser[1]
[1]*Justus Liebig Universität, Applied Microbiology, Gießen, Germany*
[2]*Justus Liebig Universität, Animal Ecology and Systematics, Gießen, Germany*
[3]*Justus Liebig Universität, Biomedical Research Center Seltersberg-Imaging Unit, Gießen, Germany*

The high concentration of microplastic particles (MPs - defined in size between 20 µm and 5 mm in diameter) in aquatic environments is a worldwide problem that has drawn considerable attention of the scientific community in the last years. However, relatively less is known about the microbial communities colonizing MPs surfaces in ocean waters and their effects on marine ecosystems. Here, we performed a laboratory scale microcosm experiment in order to characterize the bacterial colonization patterns on artificial MPs (polyethylene) and to test for specific differences of bacterial communities on MPs compared to natural particles (sediments and detritus) as well as in free-living bacterioplankton (0.22–5 µm water fraction). Sterile MP and sediment particles were incubated in parallel for twelve weeks in marine microcosms (three independent replicates). Scanning electron microscopy showed particle-specific complex eukaryotic and prokaryotic microbial communities on MPs, sediment and detritus particles. Bacterial 16S rRNA gene amplicon based PCR-DGGE community fingerprinting and Illumina sequencing revealed distinct structural and compositional patterns. MP surfaces were colonized with a specific bacterial community, whereas those occurring on natural particles were more similar to each other. The most abundant taxa on MPs were assigned to the genera *Jejudonia* (*Bacteroidetes, Flavobacteriaceae*), *Roseivivax* (*Proteobacteria, Rhodobacteraceae*) and *Marinobacter* (*Proteobacteria, Alteromonadaceae*), while sediments were strongly colonized by bacteria affiliated to the JTB255 marine benthic group (*Proteobacteria, Xanthomonadales*) and uncultured bacteria from the family *Rhodobacteriaceae* (*Proteobacteria*), detritus was dominated by bacteria classified in genera *Synechococcus* (*Cyanobacteria*), *Ruegeria* (*Proteobacteria, Rhodobacteraceae*) and *Blastopirellula* (*Planctomycetes, Planctomycetaceae*). In contrast, the free-living bacterioplankton was dominated by *Cryomorphaceae* (*Bacteroidetes*). Our data suggest that the assembly of microbial communities on particles in marine systems strongly depends on the type of particle and the respective surface. Due to the inferred specificity of bacterial communities colonizing MPs, the possibility that these artificial particles attract non-native bacterial communities is emphasized. The effect of those new bacterial assemblages on other marine organisms needs to be investigated in detail in further experiments.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

340/EEP
Dilution-to-extinction cultivation of abundant bacteria colonizing the phyllosphere of grassland exposed to elevated atmospheric CO2
O. Budich*[1], E. L. Aydogan[1], L. Anweiler[1], M. Hardt[2], G. Moser[3], C. Müller[3,4], P. Kämpfer[1], S. P. Gleaser[1]
[1]*Justus-Liebig Universität, Institut für Angewandte Mikrobiologie, Giessen, Germany*
[2]*Justus-Liebig-University, Biomedical Research Center Seltersberg-Imaging Unit, Giessen, Germany*
[3]*Justus-Liebig-University, Institute for Plant Ecology, Giessen, Germany*
[4]*University College Dublin, School of Biology and Environmental Science, Dublin, Ireland*

Global climate changes lead to increasing atmospheric carbon dioxide concentration (CO2). This will affect plants, microbial communities colonizing the aerial part of plants (phyllosphere) and the interactions among plants and phyllosphere microbes.
The aims of this study were (i) to investigate the effects of elevated CO2 on abundance and diversity of phyllosphere colonizing bacterial communities and, (ii) to cultivate the most abundant phyllosphere bacteria as model organisms for further detailed studies.
A dilution-to-extinction cultivation approach was used to study highly abundant but slow growing bacteria that can often not be targeted by cultivation on agar plates. Two abundant grassland plant species, *Arrhenatherum elatius* and *Galium album* of the Giessen Free Air Carbon Dioxide Enrichment (GiFACE) system in Linden, Germany, were selected for this study. Leaves were collected in May and August 2015 from three areas of three control (CC) and three FACE rings exposed to +20 % elevated CO2 (CE). Phyllosphere bacteria were detached mechanically in phosphate buffer, serially diluted and incubated for several weeks in liquid 0.5-fold diluted R2A medium to culture general heterotrophs. In addition, liquid mineral medium with methanol as sole carbon source was used to culture and enumerate methylotrophs by the most probably numbers (MPN) analysis. From the three highest positive dilutions cell lysates were generated, 16S rRNA gene fragments amplified and separated by denaturation gradient gel electrophoresis (DGGE). DGGE pattern were analyzed by non-metric multidimensional scaling (NMDS). Furthermore, from these dilutions bacteria were isolated and identified by partial 16S rRNA gene sequencing.
Concentrations of enriched methylotrophs and heterotrophs were in the range of 106 to 1012 MPNs g-1 fresh weight of leaves for both plant species. The concentration of heterotrophs and methylotrophs colonizing *G. album* was partially significantly lower under CE, whereas no significant differences were obtained for *A. elatius*. The NMDS analysis showed significant differences among CC and CE in the composition of abundant cultivated heterotrophs and methylotrophs from *A. elatius*. In contrast, significant differences were only obtained for heterotrophs for *G. album*. Phylogenetic identifications showed that under elevated

CO2 members of *Plantibacter*, *Pseudomonas* and *Sphingomonas* could be isolated in higher numbers than from *G. album* leaves grown under ambient CO2.
The application of the dilution-to-extinction enrichment enabled the isolation of bacteria abundant in the phyllosphere of the investigated plants species as shown by the comparison to 16S rRNA gene sequencing amplicon data. First results clearly indicated a bacterial community shifts of the phyllosphere microbiota under elevated atmospheric CO2.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

341/EEP
Root-derived H_2 drives methanogenesis in the rhizosphere of *Carex* sp.
A. Meier*[1], H. L. Drake[1], S. Hunger[1]
[1]*University of Bayreuth, Ecological Microbiology, Bayreuth, Germany*

Mires have water-saturated soils that produce the greenhouse gas methane. Vegetation and the density of plant roots in soil influences the emission of methane. For example, soil with vegetation emits more methane than soil without vegetation. Although methanogenesis is well studied in mire soils little is known about processes that drive the production of methane in the rhizosphere of mire-derived plants. Previous studies indicated that formate stimulated the formation of H_2 in the rhizosphere and that rhizospheric soil has a higher potential for methanogenesis than roots have. Thus, we hypothesized that methanogenesis in mire soil is driven by root-derived compounds. This hypothesis was addressed by anoxic microcosms with roots of *Carex* sp. and its surrounding soil set up in different ratios and by supplementation of H_2. Methane production was the highest with equal amounts of roots and soil, and decreased with decreasing amounts of soil or roots, indicating that either cell number of soil-derived methanogens or root-derived substrates decreased. The enhanced production of methane might be due to the degradation of acetate by aceticlastic methanogenesis. Production of H_2, CO_2, acetate, propionate, and butyrate increased with an increasing ratio of roots, indicating a high microbial activity of mire-derived roots. H_2 production and H_2 uptake were similar in quantities in soil-free root microcosms. In contrast, H_2 uptake was more pronounced than H_2 production in microcosms with root-free soil and stimulated the production of methane and acetate which was indicative for H_2-driven methanogenesis and acetogenesis. These results suggest that H_2 and organic carbon such as acetate are produced at the root and diffuses away into the surrounding soil where they drive the production of methane.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

342/EEP
A proteomic view at degradation of the synthetic sweetener saccharin by *Sphingobium xenophagum* strain SKN
A. Fiedler*[1], S. Oehler[1], D. Schleheck[1]
[1]*University of Konstanz, Biology, Konstanz, Germany*

The aerobic bacterium *Sphingobium xenophagum* SKN is capable of completely utilizing xenobiotic saccharin (3-sulfobenzoic acid imide) as the sole source of carbon and energy for growth. Saccharin is the oldest artificial sweetener and is used in large amounts until today. It cannot be degraded by humans and is excreted *via* the urine, suggesting that environmental bacteria play an important role in its degradation. However, any detailed information on the pathway, and on the enzymes and genes that have been recruited for assembling such a novel degradation pathway for a xenobiotic compound in strain SKN, is missing. In this work, a draft genome sequence of strain SKN was established and annotated *via* the IMG pipeline, and the genome annotation is being used as reference for a differential proteomics approaches in order to identify key enzymes/genes. Based on our preliminary results, it seems as if there are two dioxygenases involved in order to perform the initial steps of saccharin degradation. First, a multi-component Rieske-type dioxygenase system for conversion ofconverting saccharin into catechol most likely in a single reaction step, hence, concomitant with release of sulfite and ammonium. Second, a catechol 1,2-dioxygenase for aromatic ring-cleavage. Candidate genes for the two oxygenases, as well as for all enzymes of the *ortho*-ring cleavage pathway to yield acetyl-CoA and succinate, could be identified *via* differential 2D-PAGE and total proteomics. These candidate genes appear to be organised in several gene clusters, which also harbour transposase genes; the latter observation might indicate that these genes have been mobilized recently in strain SKN. The saccharin dioxygenase system candidate genes identified in this work will now be cloned and overexpressed in *E. coli*, in an attempt to confirm their predicted function.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

343/EEP

Abstract has been withdrawn.

344/EEP
Fate of chitin in freshwater sediments
S. Wörner*[1], M. Pester[1]
[1]*University of Constance, Konstanz, Germany*

Chitin is a linear polysaccharide consisting of 1,4-N-acetyl-D-glucosamine (GlcNAc) residues. It is part of the exoskeleton and cuticle of arthropods, mollusks, and worms and as such massively produced in aquatic habitats. However, no long-term accumulation has been observed in marine sediments indicating a fast and effective turnover of chitin with its degradation in freshwater habitats being little investigated [(1)(2)]. In this study, Lake Constance sediment was used to investigate anaerobic chitin turnover in a lacustrine sediment. Microcosms were set up with chitin or its monomer GlcNAc in the presence and absence of regularly spiked sulfate (500 µM) to mimic sulfate-reducing and methanogenic conditions, respectively. Carbon balance analyses under both incubation conditions indicated that at least 62% of GlcNAc and 17% of chitin were converted to acetate, CO_2 and CH_4 as the only recovered products over a period of 35 and 50 days, respectively. This translated into turnover rates of 85.6 nmol GlcNAc (g sediment f. wt.)$^{-1}$ day^{-1} and 51.1 nmol chitin (g sediment f. wt.)$^{-1}$ day^{-1} irrespective of sulfate reducing or methanogenic conditions. With both substrates, sulfate amendment had a strong impact on methanogenesis by reducing it up to 55-90%. Maximum sulfate turnover rates reached 133 nmol (g sediment f. wt.)$^{-1}$ day^{-1} in chitin and 156 nmol (g sediment f. wt.)$^{-1}$ day^{-1} in GlcNAc-amended microcosms towards the end of the incubations indicating active sulfate reducers. Under all incubation scenarios, the number of Bacteria and Archaea stayed stable throughout the incubation period with an average of 1.4 ± 1.2 × 108 16S rRNA gene copies per gram sediment (f. wt.). This indicated that the overall microbial community was not adversely affected by the addition of surplus sulfate as opposed to methanogenic conditions. The identification of microorganisms involved in chitin and GlcNAc degradation under sulfate-reducing and methanogenic conditions is currently investigated by high throughput amplicon sequencing of 16S rRNA cDNA and 16S rRNA genes. With this study, we anticipate to understand the rates of chitin degradation and its monomer GlcNAc as well as the microorganisms responsible for the degradation of this important biopolymer.

References
[1] Gooday, G.W. (1994). Physiology of microbial degradation of chitin and chitosan. *Biochemistry of microbial degradation,* pp. 279-312: Springer.
[2] Keyhani, N.O., Roseman S. (1999). Physiological aspects of chitin catabolism in marine bacteria. *Biochimica et Biophysica Acta (BBA)-General Subjects* 1473:108-22.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

345/EEP
Circum-neutral pH and Low Temperature Define *Candidatus* Nitrotoga spp. as Competitive Nitrite Oxidizer
S. Wegen*[1], E. Spieck[1]
[1]*Universität Hamburg, Microbiology, Hamburg, Germany*

So far *Candidatus* Nitrotoga spp. is known as a cold adapted nitrite oxidizing bacterium (NOB), with enrichments originating from permafrost soil of the Siberian Arctic and a cold water recirculating aquaculture system (RAS). Apart from these habitats, *Nitrotoga*-like NOB could be detected in different natural and technical environments. They are thus of importance for the global nitrogen cycle and furthermore contribute to a successful nitrification in wastewater processing. However, *Nitrospira* spp. can grow under low temperature conditions as well and they often coexist with *Nitrotoga*-like bacteria. Hence the question of niche separation between these two NOB arises.
In this study, we focused on the influence of pH on distribution of *Nitrotoga* and *Nitrospira* in co-culture. A highly enriched *Nitrotoga* from the WWTP in Hamburg-Dradenau, *Ntg.* BS, was characterized regarding its pH and temperature optimum, and subsequently combined with *Nitrospira defluvii*. Co-cultivation of both NOB was set-up in two batch bioreactors at 17°C with different pH in parallel runs using 1 mM nitrite.
Ntg. BS clearly outcompeted *Nsp. defluvii* at pH 7.4 and 17°C, correlating with its pH optimum at 7.3. Since *Nsp. defluvii* has the same pH optimum, *Ntg.* BS was probably at an advantage due to the low temperature, as it grew optimal at 17°C. At pH 6.4, no distinct predominance of either NOB was observed. However, *Ntg.* BS was never suppressed, as was *Nsp* under more adverse conditions. Thus, we could demonstrate *Ntg.* BS as a competitive NOB, dominating over *Nsp. defluvii*, when environmental parameters allow optimal growth of this cold-adapted organism. These findings confirm the importance of *Nitrotoga* spp. for nitrification in cold environments and technical applications.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

346/EEP
Searching new extremophilic microbial model systems for space exploration studies – data from a large-scale transect study in the Atacama Desert
D. Boy*[1], R. Godoy[2], G. Guggenberger[1], R. Moeller[3], J. Boy[1]
[1]*Leibniz Universität Hannover, Institut für Bodenkunde, Hannover, Germany*
[2]*Universidad Austral de Chile, Instituto de Ciencias Ambientales & Evolutivas, Valdivia, Chile*
[3]*Deutsches Zentrum für Luft- und Raumfahrt, Space Microbiology Research Group, Cologne, Germany*

The search for new model organisms for space exploration missions in the Atacama Desert is traditionally limited to a rather narrow strip (Yungay, Chile) which is believed to have the most arid conditions on Earth (McKay et al., 2003) thus harboring the most desiccation-resistant microorganisms. However, it is not clear whether Yungay is indeed the driest place in the Atacama, as this should be the one with the lowest soil organic carbon (SOC) stock and soil water (SW) content. Therefore we tested different soil samples from an aridity-gradient transect with comparable sites (inclination, position in the rain shadow of the coastal mountain range, approx. 100 km distance between the sites) spanning roughly 600 km in the Atacama Desert for SOC stocks and SW content. We found, that SOC stocks decreased with aridity from 25.5 to 2.1 kg m^{-2} cm^{-1}, while the SW contents decreased at 5 of our sites and increased in the hyper-arid zone. To our surprise, we identified one site located 100 km north of Yungay which had substantially lower SOC stocks (1.92 kg m^{-2} cm^{-1} ± 0.73) than Yungay (2.21 kg m^{-2} cm^{-1} ± 0.75), but with 0.043 g of water per 1 g of soil ± 0.03 comparable SW contents, while Yungay has 0.043 g ± 0.06. Thus we consider this site to display different growth conditions and ecological niches as compared to Yungay and therefore as promising candidate site for the identification of new species of radiation-resistant microorganisms, as the resistance against desiccation is paired with a distinct resistance to ionizing radiation due to efficient microbial DNA repair mechanisms (Mattimore et al., 1995). Soil samples were irradiated with high doses of gamma radiation up to 25 000 Gy. Surviving colonies were cultivated on a medium favoring the growth of *Deinococcus*-like species and their affiliation was determined using 16sRNA-Next Generation Sequencing. Here, we evaluate the hypothesis of ecological niching even at the most hyper-arid places of our planet on grounds of our recently identified site – with implications for the search for life in hyper-arid Martian regolith in future robotic space exploration missions such as ExoMars.

References
[1] Mattimore, V. & Battista, J. R. 1995. Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J. Bacteriol. February 1996 vol. 178 no. 3 633-637
[2] McKay, Chr. P., Friedmann, E. I., Gomez-Silva, B., Caceres-Villanueva, L., Andersen, D. T., Landheim, R. 2003. Temperature and Moisture Conditions for Life in the Extreme Arid Region of the Atacama Desert: Four Years of Observations Including the El Niño of 1997–1998. ASTROBIOLOGY. Volume 3, Number 2, 2003

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

347/EEP
Survival of the NASA Mars Odyssey isolate *Acinetobacter radioresistens* 50v1 on different spaceflight relevant antimicrobial surfaces
M. Hoef-Emden[1], R. Moeller*[1]
[1]*German Aerospace Center , Space Microbiology Research Group, Cologne, Germany*

Since many years, human mankind travels to space. One of our mayor interests is the health of astronauts and the protection of the spacecraft. Apart from external influences, the microbial burden inside of the International Space Station (ISS) may be dangerous and must be limited to a minimum. To ensure the status and the protection of the crew as well as the spacecraft itself, it is necessary to determine the survival of microorganisms on different surfaces. Microorganisms are constantly changing their strategy of survival, primarily induced by extreme environmental conditions, such as space conditions, compared to their terrestrial habitats. However, the increased levels in resistance and robustness possibly play a sensitive role in evolving new virulence factors in the space environment.
One of the bacteria on the NASA Mars Odyssey spacecraft, which have been isolated, is the Gram-negative, non-motile bacterium *Acinetobacter radioresistens*. Apart from *Deinococcus radiodurans*, *A. radioresistens* shows similar levels in radiation and oxidative stress tolerance (McCoy et al., 2012). In our work,

we used the strain 50v1, isolated from the surface of the Mars Odyssey spacecraft as well as the type strain DSM6976, which was isolated on Earth from cotton and soil samples. We investigated the resistance regarding in their desiccation tolerance on metallic surfaces including materials with different antimicrobial properties. For those experiments we exposed and desiccated both strains on the different surfaces (such as copper- and silver-containing materials) and determined the survival over different time points. First results show a high resistance of the spacecraft isolated strain compared to the type strain. These results give implications about the higher survivability of environmental microorganisms and highlight the essence of bioburden reduction and improve sterilization approaches/techniques for upcoming space exploration missions towards the search for life outside Earth.

References

[1] McCoy KB, Derecho I, Wong T, Tran HM, Huynh TD, La Duc MT, Venkateswaran K, Mogul R. 2012. Insights into the extremotolerance of *Acinetobacter radioresistens* 50v1, a Gram-negative bacterium isolated from the Mars Odyssey spacecraft. Astrobiology 12(9):854-62.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

348/EEP

Peptostreptococcaceae and _Aeromonadaceae_: Drivers of protein- and RNA-based fermentation in gut contents of the earthworm _Lumbricus terrestris_

L. Zeibich*[1], O. Schmidt[1], H. L. Drake[1]
[1]*University Bayreuth, Ecological Microbiology, Bayreuth, Germany*

By virtue of their feeding habits, earthworms are soil engineers of the terrestrial biosphere. Ingested soil-derived fermenters are conceived to be activated by the favorable conditions (e.g., anoxia and high concentrations of worm mucus-derived saccharides) in the gut of earthworms. These activated fermenters could theoretically drive the degradation of biopolymers derived from disrupted ingested plant and microbial biomass, and thus provide organic acids that could be utilized by the earthworm. The objective of this study was to resolve the capacity and identity of gut microbiota potentially linked to the degradation of biopolymers during gut passage. Anoxic microcosms of gut content of the model earthworm *Lumbricus terrestris* were supplemented with the biopolymers cellulose, xylan, protein, and RNA. Fermentation (i.e., the production of CO_2, H_2, and organic acids) was strongly stimulated in protein and RNA treatments. In contrast, fermentation was only minimally stimulated by cellulose and xylan. These results indicated that protein and RNA, rather than cellulose and xylan, are subject to rapid degradation by gut microbiota. Ilumina-based 16S rRNA and 16S rRNA gene sequencing was utilized to identify microbes potentially linked to the degradation of protein and RNA. These analyses indicated that the *Peptostreptococcaceae, Clostridiaceae*, and *Fusobacteriaceae* were primarily linked to the degradation of protein, whereas the *Aeromonadaceae* were primarily linked to the degradation of RNA. The differential stimulation of fermentative taxa with protein and RNA suggests that the engagement of biopolymer-linked fermenters in the gut is biopolymer-specific. The collective data indicated that protein and RNA, the two main soluble biopolymers released via the disruption of microbial cells in the gizzard, are subject to hydrolysis and fermentation by microbes in the alimentary canal. Thus, gut-associated fermentation of protein and RNA likely (a) contributes to the fermentation dynamics in the alimentary canal, and (b) yields important sources of organic carbon (i.e., organic acids) for both the catabolism and anabolism of the earthworm.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

349/EEP

A high-throughput-approach for the cultivation of bacterial consortia from eukaryotic hosts including a screening method for new antimicrobial compounds

J. O. Bartz*[1], S. Glaeser[1], A. Subedi[1], J. B. Mvie[1], M. Gnerlich[1], P. Schubert[2], T. Wilke[2], C. Obermeier[3], R. Snowdon[3], P. Kämpfer[1]
[1]*University of Giessen, Applied Microbiology, Giessen, Germany*
[2]*University of Giessen, Animal Ecology and Systematics, Giessen, Germany*
[3]*University of Giessen, Plant Breeding, Giessen, Germany*

A large number of bacteria and fungi have the ability to produce a variety of secondary metabolites. Some of these Natural Products are of high importance for the use in the pharmaceutical industry e.g. as antibiotics. Due to the increasing occurrence of resistant bacteria there is a high demand for the discovery of new antimicrobial compounds.

Symbiotic bacterial communities interact among each other and with eukaryotic hosts by the production of a broad range of secondary metabolites and quorum sensing molecules. Secondary metabolites often play here important roles by driving the composition of symbiotic microbial communities and protect both, the host and the host-specific microbial consortia, for pathogen invasion. For this reason, a promising strategy to find new antibiotics is the enrichment of actively interacting (metabolite producing) bacterial consortia from highly competitive habitats and screen those enrichment cultures for their antimicrobial activities.

The aim of our study was the enrichment of bacterial assemblages from natural habitats in a 96-well plate based dilution-to-extinction cultivation approach, where slow-growing bacteria are protected from overgrowth. Furthermore, not-yet-cultured or so far unculturable bacteria may be enriched in consortia in case of a co-enrichment of the special interaction partner in the microtiter plate well. In one parallel workflow, enrichment cultures were preserved for long-term storage, DNA was extracted for further analysis and a pre-screening for the production of secondary metabolites (antimicrobial compounds) and quorum sensing molecules was performed using specifically established spot-assays. Antimicrobial active enrichment cultures were differentiated at the strain level by genomic fingerprinting and phylogenetically identified by 16S rRNA gene sequencing. The results of the pre-screening and molecular identification are the bases for further tests regarding the bioactivity of the pure cultures and the bacterial consortia.

To establish this cultivation strategy, we tested both bacterial symbionts of marine corals and sponges, which are well-characterized holobionts and endophytic bacteria from rapeseed root and hypocotyl. First results indicate that the strongest inhibition of pathogenic test strains seem to appear in the presence of a co-culture with more than one strain, e.g. found for a *Pseudomonas* co-culture. Currently we investigate in more detail if single strains or only co-cultures lead to the antimicrobial activities.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

350/EEP
Effect of surface degradation of high-density polyethylene for biofilm formation
M. Erdmann[1], U. Niebergall[1], M. Böhning[2], J. Sameith *[3]
[1]*Bundesanstalt für Materialforschung und -Prüfung, Mechanik der Polymerwerkstoffe , Berlin, Germany*
[2]*Bundesanstalt für Materialforschung und -Prüfung, Technische Eigenschaften von Polymerwerkstoffen , Berlin, Germany*
[3]*Bundesanstalt für Materialforschung und -Prüfung, Biologische Materialschädigung und Referenzorganismen, Berlin, Germany*

Renewable resources become more and more relevant to maintain energy demands for an increasing global population. Biosynthetic fuels like biodiesel might replace conventional petrochemical fuels. In this study the influence of microbial growth on biodiesel and diesel in the storage tank systems were investigated. Polymeric fuel storage tanks for diesel and biodiesel provide suitable environmental conditions for a broad spectrum of fungi and various bacteria, including cyanobacteria and aerobic, heterotrophic, and even anaerobic, fermentative Bacteria [1]. However, the questions whether and how ageing of the materials is affected by biofouling processes have not been answered so far. Therefore, a model system for biofilm formation was established to study the initial attachment phase of bacteria in dependency of ageing, material quality and surface modification of thermoplastic polymers used for storage tank systems. The microbial survey is closely connected with a detailed characterization of the material"s properties and its ageing processes.
A widely applied plastic used for fuel storage tanks is high-density polyethylene (PE-HD), which is available with various additives to increase UV-light stability and even for the storage of biodiesel. These materials were aged under UV-light and temperature using a defined climate chamber set-up. Further, the repeated filling of fuels into storage tanks was mimicked, as this has an important impact on the life-cycle length for the storage container [2]. The aged polymers showed a significant change in the material"s characteristics, including surface characteristics (e.g. hydrophobicity) and carbonyl groups. The effect of the changed material properties on the biofilm formation are studied using bacterial isolates, previously obtained from a "dieselpest" [3], and as a model-reference *E. coli*. The unaged polymers showed already some significant differences for the initial attachment of *E. coli* K12. Polyethylene with additives was colonized faster than the reference material (without additives) although the final biofilm coverage was not impaired. The settlement of one kerosene-isolate (*Bacillus* species) on the unaged materials independently of additives was only minor interfered.
In future, our results should give stakeholders in industry and public authorities a better estimation of the life-cycle security for fuel storage tank systems and on the fuel quality. Further, our results could help to test and develop new materials or additives to prevent biofouling processes.

References
[1] Restrepo-Florez J. M., *et al.* (2014) in Fuel, 128: 240-247.
[2] Böhning M., *et al*. (2014) in Polymer Testing, 34: 17–24.
[3] Martin-Sanchez P. M., *et al.* (2016) in Biofouling, 32: 635-644.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

351/EEP
The impact of thermophile sulfate-reducing bacteria on serpentinization reactions
S. Günther[1], E. Schwarzenbach[2], J. Sameith *[1]
[1]*Bundesanstalt für Materialforschung und -Prüfung, Biologische Materialschädigung und Referenzorganismen, Berlin, Germany*
[2]*Freie-Universität Berlin, Institut für Geologische Wissenschaften, Mineralogie-Petrologie, Berlin, Germany*

Serpentinization is an important process in the Earth`s oceanic lithosphere, where the reaction of olivine und pyroxene with water forms serpentine and magnetite. During this alteration reaction gaseous hydrogen is produced abiogenically, which has been inferred as an essential energy source for microbes living in these systems. The serpentinization reaction directly influences the chemical composition of the lithosphere and the ocean chemistry and, thus, connects the lithosphere, hydrosphere and biosphere [1]. While the geochemical processes are rather well studied in these systems, the biological influence on the processes is not well understood. However, microbial activity has been documented for serpentinization systems, e.g. along the Mid-Atlantic Ridge [2], in surficial carbonate formations constraining the activity of sulfate-reducing bacteria and methanogenic archaea. The question arises whether microbial populations can be sustained by the hydrothermal alteration of the oceanic lithosphere and whether the microbial populations directly influence and accelerate the serpentinization process.
For our experimental setup the surface of mono-mineral cubes of olivine and pyroxene are directly exposed to sulfate-reducing bacteria, using an anaerobic semi-continuous fermenter system. Besides mesophilic sulfate-reducing Bacteria of the *Desulfovibrio* genus also thermophilic sulfate-reducing enrichment cultures from geothermal, saline fluid systems were included to investigate the mineral surface alteration. The mineral alteration was monitored using electron microprobe analysis, scanning electron microscopy and isotope fractionation of sulfide [3], while biofilm formation was documented and sulfate reduction activity measured.

References
[1] Scambelluri M.*, et al.* (2016), Earth Planet Sc Lett, 441: 155-166.
[2] Klein F.*, et al.* (2015), P Natl Acad Sci USA, 112: 12036-12041.
[3] Schwarzenbach E.M.*, et al.* (2012), Geochim Cosmochim Ac, 91: 283-305.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

352/EEP
Dissemination of antibiotic resistant bacteria and antibiotic resistance proteins in wastewater assessed by metaproteomic
D. Zühlke*[1,2], V. Kulow[1], D. Schneider[3], R. Daniel[3], K. Riedel[1,2]
[1]*Ernst-Moritz-Arndt-University, Institute of Microbiology, Greifswald, Germany*
[2]*Institute of Marine Biotechnology, Greifswald, Germany*
[3]*Georg-August-University, Institute of Microbiology and Genetics, Göttingen, Germany*

Introduction: The massive use of antibiotics to treat infections in humans and animals is leading to a growing input of antibiotics into the environment and consequently the selection of antibiotic-resistant bacteria. Urban wastewater treatment plants (UWTP) have been considered as a hotspot for the dissemination of antibiotic-resistant bacteria. However, despite a growing interest in the role of UWTP in the spread of antibiotic-resistant bacteria and the dissemination of resistant determinants, only limited quantitative data is available investigating this process at different stages of the wastewater treatment. Furthermore, seasonal changes

of antibiotic-resistant bacteria and resistance genes/proteins have not been studied very detailed so far.
Objectives: Using a metaproteomics approach, we aim at the identification and quantification of metabolically active and antibiotic-resistant bacteria at different sites of the UWTP. The metaproteome data will be complemented by metagenomic and metatranscriptomic analyses. A special emphasis will be put on the analysis and comparison of the microbial community and proportion of antibiotic-resistant microorganisms comparing the influent and effluent of the UWTP.
Methods & results: Composite samples from different stages of the local wastewater treatment plant were collected. In a first step, protein extraction protocols were tested for their applicability on wastewater samples and the extracted proteins were analyzed by GeLC-MS/MS. The composition of the microbial wastewater community was analyzed by fluorescence in situ hybridization (FISH) and plating on selective media for differentiation of taxonomic groups. Additionally, the proportion of antibiotic-resistant microbes within the bacterial community was calculated using antibiotic-containing agar plates. First quantitative proteome data will be presented describing the bacterial community structure and function at different sites of the wastewater treatment plant. The results from the metaproteome analysis will be supported by culture-dependent and culture-independent molecular methods.
Conclusion: Metaproteomics are an ideal tool to target the metabolically active antibiotic-resistant bacterial community. To assess the risk of antibiotic-resistant bacteria in wastewaters and to prevent their spread into the environment insights into their dynamics during treatment in UWTP are crucial.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

353/EEP
Genome-based differential proteomic analysis of the marine, nutritionally versatile, sulfate-reducing bacterium, *Desulfosarcina variabilis* DSM 2060
M. Dörries[1], L. Wöhlbrand[1], V. Schnaars[1], C. Hinrichs[1], D. Thies[2], M. Kube[3], R. Reinhardt[4], R. Rabus*[1]
[1]*Carl von Ossietzky Universität Oldenburg, ICBM- General and Molecular Microbiology, Oldenburg, Germany*
[2]*Max Planck Institute for Marine Microbiology, Bremen, Germany*
[3]*Johann Heinrich von Thünen Institute, Institute for Forest Genetics, Waldsieversdorf, Germany*
[4]*Max Planck Genome Centre Cologne, Köln, Germany*

Question: Sulfate-reducing bacteria (SRB) are key players of the carbon- and sulfur-cycles in the sediments of the worlds oceans. Of prominent habitat relevance are members of the *Desulfosarcina-Desulfococcus* clade within the deltaproteobacterial family of *Desulfobacteraceae*. A metabolically versatile representative of this cluster is *Desulfosarcina variabilis* DSM 2060 that completely oxidizes a large variety of organic acids (up to C14) as well as aromatic compounds under anoxic conditions, and has also the capacity of chemolithoautotrophic growth (with H2 and CO2). In the present study proteogenomics was applied to reconstruct the metabolic network of *D. variabilis*.
Methods: Manual revision of automatically predicted and annotated protein-coding sequences (CDS) was combined with differential proteomic data (2D-DIGE, shotgun, membrane protein-enriched fraction) generated from cells adapted to 28 (13 aromatic and 15 aliphatic compounds) different substrate conditions to allow for improved functional prediction.
Results: With a genome size of 9.64 Mbp and 8579 predicted CDS *D. variabilis* holds one of the largest prokaryotic genomes, markedly exceeding those of all currently genome-sequenced SRB. The comprehensive proteogenomic dataset allowed for reconstructing the three major metabolic modules of *D. variabilis*: (i) heterotrophy (i.e. degradation pathways for aromatic and aliphatic compounds), (ii) chemolithoautotrophy and (iii) and energy metabolism centering on dissimilatory sulfate reduction. In case of heterotrophy, peripheral degradation routes feed via central benzoyl-CoA, (modified) b-oxidation and methylmalonyl-CoA pathways into the Wood-Ljungdahl pathway for complete oxidation of acetyl-CoA to CO2. Dissimilatory sulfate reduction is embedded in a complex electron transfer network composed of cytoplasmic components (ETFs and electron bifurcating Hdr/Mvh and Nfn complexes) and diverse membrane complexes (e.g. Dsr, Qmo, Hmc, Tmc, Qrc and Rnf).
Conclusions: First comparative analyses shed light in genome congruencies and differences among members of the *Desulfobacteraceae*. Overall, this study contributes to a comprehensive proteogenomic understanding of the habitat - relevance and -success of the deltaproteobacterial SRB family *Desulfobacteraceae*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

354/EEP
Development of a bioelectrochemical system for the removal of ammonia from wastewater
J. Heise*[1], N. Heyer[1], R. U. Meckenstock[1], L. Steuernagel[2], M. Denecke[2]
[1]*University of Duisburg-Essen, Aquatic microbiology, Essen, Germany*
[2]*University of Duisburg-Essen, Siedlungswasser- und Abfallwirtschaft, Essen, Germany*

Background: Removal of ammonia from municipal wastewater is essential for preventing oxygen depletion of rivers due to ammonia oxidation or fertilization. In wastewater treatment plants, ammonia is thus oxidized via nitrification and removed via subsequent denitrification steps or a combination of nitrification and anammox.
Objectives: Here, we want to establish a complete removal of ammonia from wastewater by applying bioelectrochemical systems, where conditions can be precisely controlled. As the first step, we want to oxidise ammonia to nitrite or nitrate by nitrifying bacteria which obtain molecular oxygen from an electrolysis reaction at an anode. The electrolysis shall allow for high microbial nitrification rates while keeping dissolved oxygen concentrations low.
Methods: Nitrifying bacteria were cultivated on stainless steel felt in a nitrification reactor for wastewater treatment. The felts covered with biofilms were then introduced into bioelectrochemical reactors. The oxygen production rates as well as the ammonia removal rates were determined at different anodic electrode potentials.
Results: The results of our study show that oxygen production rates are constant at a potential of 1.10 V vs. Ag/AgCl, while ammonia oxidation rates are increasing. The concentrations of ammonia, nitrite and nitrate in the reactors indicate a complete nitrification to nitrate.
Conclusion: The oxygen production and ammonia removal rates determine the range of potentials in which complete oxidation of ammonia can take place without inhibiting the microbiota due to oxygen depletion or excessive concentrations of oxygen.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

355/EEP
Crude oil degradation potential at elevated pressure by a *Rhodococcus* strain isolated from the Gulf of Mexico
S. Hackbusch*[1], J. Viamonte[1], N. Noirungsee[1], P. Bubenheim[1], X. Sun[2], J. Kostka[2], A. Liese[1]
[1]*TUHH, Institute of Technical Biocatalysis, Hamburg, Germany*
[2]*Georgia Institute of Technology, School of Biology and Earth & Atmospheric Sciences, Atlanta, Georgia, United States*

Up to 25% of the hydrocarbons spilled into the Gulf of Mexico after the Deepwater Horizon oil spill in 2010 were presumably consumed by microorganisms. The composition of a persistent plume between 900-1300 m depth was characterized by high concentrations of polycyclic aromatic hydrocarbons (PAHs), medium length alkanes, methane and other gases. Further oxygen anomalies were detected which are believed to result from oxidative processes caused by the indigenous microbial community.
Most recent lab-based research to investigate microbial hydrocarbon degradation under deep sea conditions neglected the effect of elevated pressure on microbial performance. We incubated the model strain *Rhodococcus* PC20, which was isolated from sediment in the vicinity of the spill site, at 150 bar (corresponds to 1500 m depth). Experiments indicate a pressure tolerance of this strain concerning growth and oxygen consumption. However the initial oxidation of hydrocarbons is hindered at elevated pressure and thus suggests an altered usage of degradation pathways during high pressure incubation.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

356/EEP
Comparing microbial diversity in biogas and sewage treatment plants by 16S amplicon sequencing
C. Büttner*[1], M. Noll[1]
[1]*Hochschule für angewandte Wissenschaften Coburg, Coburg, Germany*

Since the Renewable Energy Act amendment of 2014 operators of biogas plants (BP) are interested to increase alternatives to renewable resources (RR) as substrates. Likewise sewage treatment plants (STP) have stricter regulations of the disposal of fermentation residues. Both plants are based on similar microbial processes of biogas production and therefore a comprehensive analyses of the microbial community composition of many plants is needed. As microbial communities of BP with RR are described in detail, a lack of knowledge is present for plants with alternatives to RR and its correlation to plant data such as process temperature, pH and substrate basis. The aim of this study was to compare the microbial community composition of a variety of biogas plants (15 plants) using only or mainly alternatives to RR and of sewage treatment plants (10 plants). The genomic DNA of biogas and sewage sludge was extracted using a modified phenol-chloroform-extraction (STP) or a CTAB-based method (BP). Extracted DNA was quality tested and sequenced by the MiSeq-technology using the 515F and 806R primer set for simultaneous amplification of bacterial and archaeal 16S rRNA gene. Sequencing results of biological replicates were similar to each other indicating a high sequencing reproducibility. Rarefaction analysis showed a phylogenetic coverage on order level. BPs and STPs differed significantly in both operation taxonomical units (OTU) composition and relative abundancies. STPs had a significant ($p=0.05$) higher abundance of the domain *Archaea* compared to BP. Moreover, Shannon-Index in STPs was significantly ($p=0.05$) higher in relation to BPs. Substrate basis and temperature seems to be the most important factor for community composition. Correspondence analyses of BPs visualized several clusters. One cluster consists of two plants mainly using cattle manure under mesophilic conditions as main substrate. Members of different OTUs belonging to the order *MBA08* and *Bacteroidales* were found in higher abundancies than in other BPs. Another cluster using food wastes at thermophilic temperatures was composed by high fractions of members of the orders SHA-98 and *Thermotogales*. Correspondence analysis of STPs resulted in various clusters. One cluster consisting of three STPs showed lower abundancies of an OTU of order *Synergistales* and higher abundancies of an OTU belonging to order SHA-98. Another cluster can be described by high abundancies of OTUs belonging to order *Spirochaetales* and *Thermotogales*. Further data analysis will show whether there are more correlations between plant parameters and resulting microbial communities.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

357/EEP
Freshwater sponges – a yet unexplored resource for novel talented producers
T. Kohn*[1], P. Rast[1], O. Jeske[1], B. Förster[2], A. Heuer[1], S. Wiegand[1], F. Surup[2], F. Brümmer[3], R. W. Müller[3], M. Stadler[2], C. Jogler[4], M. Jogler[1]
[1]*Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[2]*Helmholtz Centre for Infection Research, Braunschweig, Germany*
[3] *University of Stuttgart, IBBS, Stuttgart, Germany*
[4]*Radboud University, Nijmegen, Netherlands*

Sponges (Porifera) are a rich source of novel bioactive compounds, which are mainly produced by their highly complex microbiota. While previous studies mainly focused on marine sponges and their microbial and biotechnological repertoire, sponges from freshwater habitats are mainly unexplored. Thus, in our work we focused on freshwater sponges from different German lakes. To address the microbial diversity of these exceptional habitats, we applied cultivation independent 16S rRNA amplicon sequencing along with high-throughput cultivation. Since cultivation of interesting talented producers is a challenging task, we designed novel targeted media. Novel strains obtained have been screened subsequently towards their potential to produce novel molecules with bioactive potential. Afterwards the structure of novel small molecules has been elucidated. Here we present the first results and the proof of our concept. First, we discovered multiple novel strains from the yet untapped freshwater sponges. Along other exciting novel genera such as members of the Phylum Gemmatimonadetes we for example found multiple novel planctomycetal strains and exemplarily characterized two isolates from limnic and marine sponges in detail. Their genome sequence revealed the potential to produce small molecules. In addition, we elucidated the first structures of planctomycetal secondary metabolites. Our ultimate goal is to reveal the microbiota of freshwater sponges, to screen them for bioactive compounds and to unearth their manifold bioactive potential.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

358/EEP
Tracking down patterns of long-distance electron transfer by filamentous bacteria in flooded soils and groundwater sediments
C. Sachs*[1], D. Kanaparthi[1], T. Lüders[1]
[1]*Helmholtz Zentrum München, Institute for Groundwater Ecology, München, Germany*

The discovery of cable bacteria in marine sediments, capable of coupling spatially separated redox processes via long-distance electron transfer (LDET) over cm-distances, has revolutionized our perspective of microbial activities at redox gradients. Also in contaminated groundwater sediments, primary evidence for the

existence of such filamentous, sulfide-oxidizing *Desulfobulbaceae* has recently been reported (1). However, a more comprehensive understanding of the diversity and ecology of bacteria capable of LDET in terrestrial sediments is still at lack. Also in flooded soils, such populations can be hypothesized to occur, at least transiently upon water saturation after heavy rainfall. To follow up on this, more efficient enrichment and laboratory investigation strategies for terrestrial LDET bacteria are clearly needed, which is the objective of this ongoing work. Here, we introduce the use of a new gradient cultivation strategy in laboratory columns filled with saturated soil or sediment, including a central agar pillar. The columns filled with soil or sediment, water saturated and incubated at room temperature for 6 weeks in dark. The central agar pillar was originally introduced as a diffusion space to facilitate microsensor measurements of the depth profiles of oxygen, pH and sulfide. This strategy may circumvent the problematics of microsensor measurements in mineral sediments and structured soils. And indeed, microsensor profiling indicated the development of an oxygen-sulfide interphase at a depth of 2-3mm within a few days of incubation. As expected for LDET, the hypoxic zone between the redox counter-gradients widened during incubation and the formation of iron crusts was observed. Surprisingly, microscopic observation of the agar pillar itself revealed it to be densely colonized by filamentous bacteria. These filaments were 0.5µm in width and extended up to 1cm in length, closely resembling marine cable bacteria. All the filaments were found in the sulfide depleted zone extending up to 1 cm below the oxic-anoxic interface. No filaments were found in the deeper layers of agar or above the oxic-anoxic interface. A detailed characterization of these potential LDET bacteria in the columns by FISH and community sequencing is currently ongoing. The results of our study suggest that LDET populations may be readily detectable in groundwater sediments and flooded soils via our new agar pillar gradient cultivation strategy. Although filamentous *Desulfobulbaceae* have been reported from a number of sedimentary environments, effective enrichment strategies or pure cultures are not available to date, impeding a thorough ecophysiological characterization of these enigmatic bacteria.

References

[1] Müller H, Bosch J, Griebler C, Damgaard LR, Nielsen LP, Lueders T & Meckenstock RU (2016) Long-distance electron transfer by cable bacteria in aquifer sediments. ISME J doi: 10.1038/ismej.2015.1250.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

359/EEP

Impact of moderate temperature changes on *Neisseria meningitidis* adhesive phenotypes and proteome

M. Lappann[1], A. Otto[2], M. Brauer[1], D. Becher[2], U. Vogel[1], K. Johswich*[1]
[1]*University of Würzburg, IHM, Würzburg, Germany*
[2]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*

Introduction: Although *N. meningitidis* are feared for their capacity to cause life-threatening disease, they commonly inhabit the human upper respiratory tract without causing symptoms. Since the human nasopharynx is the only niche in which meningococci persist, it would be enticing to believe that these bacteria do not require mechanisms to adapt to changes in environmental factors other than the hosts immune response. However, the temperature in the nasopharynx is coupled to that of the inhaled air, therefore, *Neisseria meningitidis* must face temperature changes depending on the ambient air temperature. Indeed, the nasopharyngeal temperature can be substantially lower than 37°C, the temperature which is commonly used in experimental settings.

Objective: The purpose of this study was to compare the differential phenotypes and proteomes between meningococci grown at standard laboratory conditions (37°C) and meningococci grown at a more realistic average nasopharynx temperature (32°C).

Methods: Meningococci were grown at 37°C or 32°C and correlates of adhesive properties such as biofilm formation, autoaggregation and cellular adherence to FaDu and Detroit cells were assessed. Furthermore, comparative proteome analysis based on metabolic labelling with ^{15}N in combination with tandem mass spectrometry was used to define differentially expressed proteins between both temperatures.

Results: When grown at 32°C, *N. meningitidis* showed increased biofilm formation, autoaggregation as well as adhesion to epithelial cells. Proteome analysis revealed differential protein expression levels between 32°C and 37°C, predominantly affecting the bacterial envelope. Among 375 analyzed proteins, 49 were localized in the outer membrane, 21 in inner or outer membrane, 35 in the periplasm, 56 in the inner membrane and 208 in the cytosol; a further 6 proteins could not be spatially assigned. The outer membrane proteins NHBA, NMB1030 and ACP showed strongest upregulation at 32°C and were partially responsible for the observed temperature-dependent phenotypes. Screening of different global regulators of *Neisseria meningitidis* revealed that the extracytoplasmic sigma factor, σE, might be involved in the temperature-dependent biofilm formation.

Conclusion: Meningococci show significantly enhanced adhesive properties at 32°C, which generally makes this a more suitable temperature in experimental setups. The data indicate that subtle temperature changes trigger adaptation events promoting mucosal colonization by meningococci. This could be interesting with respect to seasonal patterns of meningococcal transmission and disease.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

360/EEP

Partial genomes of uncultured Planctomycetal and Verrucomicrobial Species from Kelp biofilm metagenomes

J. Vollmers*[1], M. Frentrup[1], P. Rast[1], C. Jogler[1], A. K. Kaster[1]
[1]*Leibnitz Institute DSMZ, Junior Research Group Single Cell Genomics, Braunschweig, Germany*

The kelp forest of the Pacific temperate rocky marine coastline of Monterey Bay in California is a dominant habitat for large brown macro-algae in the order of *Laminariales*. It is probably one of the most species-rich, structurally complex and productive ecosystems in temperate waters and well studied in terms of trophic ecology. However, still little is known about the microorganisms thriving in this habitat. A growing body of evidence suggests that microorganisms associated with macro-algae represent a huge and largely untapped resource of natural products with chemical structures that have been optimized by evolution for biological and ecological purposes. We here analyzed biofilm samples from the brown macro-algae *Macrocystis pyrifera* sampled in November 2014 in the kelp forest of Monterey Bay by a metagenomic shotgun approach, focusing on the PVC superphylum. Although not very abundant, we were able to reconstruct partial genomes of novel Planctomycetal and Verrucomicrobial species with no cultured representatives. The found genomes harbor secondary metabolite clusters, contributing to our hypothesis that through inter species interaction, microorganisms might have a substantial effect on kelp forest wellbeing and/or disease-development

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

361/EEP

Engineering *Pseudomonas putida* KT2440 as biocatalyst for the conversion of lignocellulose hydrolysates

Y. Wang*[1], F. Horlamus[2], M. Henkel[2], R. Hausmann[2], A. Wittgens[1], F. Rosenau[1]
[1]*Center for Peptide Pharmaceutials, Ulm University, Ulm, Germany*
[2]*Institute of Food Science and Biotechnology, University of Hohenheim, Department of Bioprocess Engineering (150k), Stuttgart, Germany*

Introduction: Lignocellulosic materials are the most abundant and renewable resource in the world, it contains cellulose, hemicellulose and lignin. The hydrolysates from lignocellulose are mainly composed of hexoses (D-glucose, D-mannose, D-galactose) and pentoses (D-xylose, L-arabinose), as well as organic acids. Although the sugar composition can vary among different biomass sources, the pentose fraction is of relevant importance (xylose 5-20%, arabinose 1-5%). Consequently, the high-sugar- level hydrolysates have a good potential to be carbon sources for the growth of microorganisms. However, the utilization of pentose sugars presents a big challenge for most of the microbes.
Objectives: This study aims at the construction of metabolic engineered strains for the efficient utilization of pentose sugars. Therefore, the genetically modified microorganisms "biocatalysts" have the potential to make lignocellulosic materials a substrate of enormous biotechnological value.
Materials & Methods: *Pseudomonas putida* KT2440 was used as the host strain. The genes *xylA* (xylose isomerase) and *xylB* (xylulose kinase) from *E.coli* DH5α were introduced into the host strain as well as the genes *araA* (L-arabinose isomerase), *araB* (L-ribulokinase) and *araD* (L-ribulose-5-phosphate 4-epimerase) from *E.coli* K12-MG1655, obtaining a xylose metabolizing strain (*P. putida* KT2440_*xylAB*) and an arabinose metabolizing strain (*P. putida* KT2440_*araBAD*). The growth of the two strains in each single sugar of glucose, xylose, arabinose and different combinations of the mixtures were investigated by measuring cell density (OD_{600}). Furthermore, the possibility of using hydrolysates as a substrate for recombinant *P. putida* KT2440 strains was evaluated.
Results: It was surprisingly found both *P. putida* KT2440_*xylAB* and *P. putida* KT2440_*araBAD* were able to grow on xylose and arabinose with a high cell densities and growth rates comparable to cultivation with glucose. *P. putida* KT2440_*xylAB* started growing earlier on xylose than on arabinose and finally reached a higher OD. In contrast *P. putida* KT2440_*araBAD* showed better growth on arabinose than on xylose with higher OD and growth rate. On the mixtures of sugars, the obtained curves demonstrated that all sugars were efficiently consumed, but glucose was always utilized first. In addition, *P. putida* KT2440_*xylAB* preferably consumed xylose and *P. putida* KT2440_*araBAD* preferably consumed arabinose.
Conclusion: We successfully developed biocatalysts for pentose utilization, which is a promising platform for biotechnological applications based on lignocellulosic biomass. Regarding the interesting phenomenon that xylose and arabinose can be metabolized by both strains, we hypothesize the enzymes expressed in the pathways have nonspecific activities, which is currently under investigation.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

362/EEP

Indo-Pacific corals under a climate change scenario - Impact of increased water temperature and decreased pH on coral microbiomes

J. Schellenberg*[1], S. Petrov[1], A. Franco[1], P. Schubert[2], P. Kämpfer[1], T. Wilke[2], S. P. Glaeser[1]
[1]*Justus-Liebig-Universität Giessen, Angewandte Mikrobiologie, Giessen, Germany*
[2]*Justus-Liebig-Universität Giessen, Tierökologie und Spezielle Zoologie, Giessen, Germany*

Global climate change, particularly increased water temperatures and decreased pH, threatens coral reefs, which harbor the highest marine biodiversity. The persistence of these ecosystems may depend on the resilience of the coral holobiont, i.e., the host and its microbial symbionts. Despite numerous studies, the impact of ocean acidification and increased water temperatures on coral holobionts is still not fully understood. The aim of this study was to investigate microbiome shifts of the most abundant associated bacteria and zooxanthellae of major reef-building corals under a global change scenario. Three scleractinian corals, *Acropora tumida*, a *Montipora* sp., and *Pocillopora damicornis*, were exposed for 8 weeks to a decreased water pH (0.4 pH units below the pH in control aquaria) and a water temperature increase by 1.2 °C (control: 27.5 °C, pH 8.1). Effects on microbial communities were investigated by comparing denaturing gradient gel electrophoresis (DGGE) community fingerprints at the beginning of the experiment (t0) with those obtained after 8 weeks of incubation (t1). DGGE analysis was performed with universal primers amplifying the bacterial 16S rRNA gene and the eukaryotic 5.8S and 28S rRNA gene internal transcribed spacer 2 (ITS2), respectively. All coral fragments survived the treatments without visible signs of stress. Rather uniform but highly diverse bacterial community patterns were detected for each coral, while the associated zooxanthellae types were uniform as well, but species specific. At decreased pH mostly changes regarding the relative abundance of bacterial taxa were observed but overall 7 of 12 host fragments (58%) switched their dominant zooxanthellae type. Increased temperature had no effect on zooxanthellae communities, but provoked shifts within the bacterial community especially for the *Montipora* sp. and *P. damicornis*. In summary, the experiment showed rather stable microbial communities for corals in a short time climate change experiment. However, changing environmental conditions affect coral holobionts differently. In this study, *Montipora* sp. seemed to be slightly more resilient than *A. tumida* and *P. damicornis*. Survival of coral reefs depends on the adaptation capacity of coral holobionts, which may be significantly controlled by the coral microbiomes is crucial in times of rapidly changing environmental conditions.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

363/EEP

A third type of degradation of the plant sugar sulfoquinovose in *Bacillus aryabhattai* strain SOS1

B. Frommeyer*[1], A. Fiedler[1], S. Oehler[1], D. Schleheck[1]
[1]*Universität Konstanz, Department of Biology, Konstanz, Germany*

Sulfoquinovose (SQ, 6-deoxy-6-sulfoglucose) is a prominent natural organosulfonate and part of the plant sulfolipid sulfoquinovosyl diacylglycerol (SQDG). SQ is produced essentially by all phototrophic organisms and is, thus, a relevant component of the biogeochemical sulfur cycle. Recently, we demonstrated two bacterial degradation pathways for SQ. First, a sulfoglycolytic pathway in *Escherichia coli* K-12 [1], in which SQ is catabolized to 2,3-dihydroxypropane-1-sulfonate (DHPS) in direct analogy to the Embden-Meyerhoff-Parnas pathway. Second, a pathway in *Pseudomonas putida* SQ1 [2], in which SQ is catabolized to 3-sulfolactate (SL) in direct analogy to the

Entner-Doudoroff pathway. The excreted DHPS and SL can then be utilized completely by other bacteria, concomitant with release of sulfate [3]. Now, we explore another type of SQ pathway in a *Bacillus* strain, which excretes SL, but not DHPS, during growth with SQ. Genome sequencing and differential proteomics indicated that, among others, a transaldolase enzyme and, thus, a pathway with some analogy to the pentose phosphate pathway might be involved. Currently, we are aiming at producing these enzymes heterologously in order to confirm a predicted third type of pathway for SQ, and will present our preliminary results.

References

[1] Denger, K., et al., *Sulphoglycolysis in Escherichia coli K-12 closes a gap in the biogeochemical sulphur cycle.* Nature, 2014. 507(7490): p. 114-7.
[2] Felux, A.-K., et al., *Entner–Doudoroff pathway for sulfoquinovose degradation in Pseudomonas putida SQ1*. Proceedings of the National Academy of Sciences, 2015. 112(31): p. E4298-E4305.
[3] Denger, K., et al., *Sulfoquinovose degraded by pure cultures of bacteria with release of C3-organosulfonates: complete degradation in two-member communities.* FEMS Microbiol Lett, 2012. 328(1): p. 39-45.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

364/EEP

Heme-*b* containing Lcp1$_{VH2}$ of *Gordonia polyisoprenivorans* VH2

S. Oetermann*[1], A. Steinbüchel[1]
[1]*Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*

Production of large amounts of rubber materials lead to challenges in terms of recycling used rubber. For an economically friendly recycling method, the biological polyisoprene-degradation could be of interest. In addition to some fungi, many Gram-positive actinobacteria and a few Gram-negative bacteria are capable of degrading this persistent polymer. *Gordonia polyisoprenivorans* VH2 is a model organism, which metabolizes this polymer [poly(*cis*-1,4-isoprene)] as sole source of carbon and energy in an efficient way, compared to other bacteria. The key enzyme of the catabolic pathway, the latex clearing protein (Lcp1$_{VH2}$), catalyzes the initial step: the oxidative cleavage of the polymer into smaller oligomers (1). Via LC-ESI-MS, spectral analyses and detection of a peroxidase activity of the native enzyme, it was shown that Lcp1$_{VH2}$ contains a non-covalent bound heme-*b* as a cofactor. Hitherto, the successful purification of two Lcps (in addition to Lcp1$_{VH2}$) is described: Lcp$_{K30}$ (from *Streptomyces* sp. K30) and Lcp$_{Rr}$ (from *Rhodococcus rhodochrous*), which both contain as well heme-*b* as cofactor (2, 3). Further studies with Lcp1$_{VH2}$ were performed to predict amino acid residues responsible for the coordination of heme-*b*. Interestingly, Lcp1$_{VH2}$ is predicted to contain a domain, to which no function can be assigned up to now (domain of unknown function, DUF2236). More than 50 amino acid sequences with high similarities towards Lcp1$_{VH2}$ show the same DUF2236, in which three histidines (H195, H200, and H228) are conserved in all sequences. Hence, all three histidines were replaced by alanines in Lcp1$_{VH2}$ in a site-directed mutagenesis. The influence of these changes was then verified by complementation experiments. The rubber-negative double deletion mutant wherein both *lcp*-homologs were deleted, was complemented with *lcp1*$_{VH2}$H195A, *lcp1*$_{VH2}$H200A, and *lcp1*$_{VH2}$H228A using the shuttle vector pNC9501. Only the mutant with exchanged amino acid at position 195 was not able to grow on rubber or to mineralize it. The influence of amino acid change H195A in *in vivo* rubber degradation has been tested for the first time. Further purification and *in vitro* analyses of recombinant, stable Lcp1$_{VH2}$H195A confirmed the loss of function which could be ascribed to the loss of heme, which became evident by the loss of the red color and spectral analyses. H195 is likely to be involved in the coordination of the heme-*b* in Lcp1$_{VH2}$.

References

[1] Hiessl S, Böse D, Oetermann S, Eggers J, Pietruszka J, Steinbüchel A. 2014. Appl Environ Microbiol 80:5231–40.
[2] Birke J, Röther W, Jendrossek D. 2015. Appl Environ Microbiol 81:3793–99.
[3] Watcharakul S, Röther W, Birke J, Umsakul K, Hodgson B, Jendrossek D. 2016. BMC microbiology 16:92.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

365/EEP

Microbial degradation of Ibuprofen in the hyporheic zone of a lowland stream

C. R. Njeru*[1], M. Posselt[2], M. A. Horn[3]
[1]*Universität Bayreuth, Ecological Microbiology, Bayreuth, Germany*
[2]*Stockholm University, Department of Environmental Science and Analytical Chemistry, Stockholm, Sweden*
[3]*Leibniz Universität Hannover, Institute of Microbiology, Hannover, Germany*

Ibuprofen is a non-steroidal anti-inflammatory pain reliever and among pharmaceutical residues detected in aquatic environments. Widespread use of the drug coupled with incomplete removal during wastewater treatment results in its persistence in effluents and receiving waters. Potential total removal by microbial activity in the hyporheic zone (HZ) of rivers downstream of wastewater treatment plant discharge sites has been hypothesised. Microbial communities associated with ibuprofen degradation are essentially unknown. To address this hypothesis, two sets of oxic HZ sediment microcosms spiked exclusively with ibuprofen (5, 40, 200 and 400 µM), or ibuprofen (5, 40, 200 and 400 µM), and 1 mM acetate were set up under laboratory conditions. Ibuprofen degradation in non-sterile sediments relative to autoclaved sediments indicated removal by microbial degradation. Ibuprofen was completely consumed in the absence and presence of supplemental acetate after approximately 11 and 16 days, respectively. Refeeding of ibuprofen and acetate in the respective setups after the initial depletion resulted in complete degradation within 24 hours in all treatments. Metabolites of ibuprofen included 1-, 2-, 3-Hydroxy- and Carboxyibuprofen. Quantitative real-time PCR revealed no pronounced differences in copy numbers of 16S rRNA gene or transcripts between non-spiked controls and treatments. Time-resolved triplicate amplicon Illumina MiSeq sequencing targeting the 16S rRNA genes and transcripts revealed increased relative abundances of operational taxonomic units affiliating with *Proteobacteria*, *Acidobacteria, Actinobacteria* and *Firmicutes* in treatments with rather than without ibuprofen. *Alpha-*, *Beta-* and *Deltaproteobacteria* were most active as indicated by RNA-based analyses. The collective results indicated that (i) HZ sediments are capable of ibuprofen removal and (ii) more diverse microbes than previously thought including the genera *Fodinicola*, *Hyphobacterium*, and members of *Acidobacteria* subdivision 6 are associated with such an ecosystem service.

Key words: Ibuprofen, persistence, hyporheic zone, amplicon illumina sequencing, micropollutant

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

366/EEP
Active microorganisms in oil reservoirs
M. Pannekens*[1], A. Meier[1], R. U. Meckenstock[1]
[1]*Biofilm Centre, University Duisburg-Essen, Aquatic Microbiology, Essen, Germany*

Background: The present paradigm for microbial degradation in oil reservoirs is that microorganisms are active at the oil-water-transition-zone leading to the formation of heavy oil or bitumen. The "Pitch Lake" is the biggest natural bitumen seep, located in Trinidad & Tobago. Here we found that oil degrading microorganisms can also thrive in tiny water droplets (1-10 µl) embedded in the oil body itself.
Objectives: So far it could be shown that these small water droplets in the oil contain microorganisms. However, we do not know yet what the population density is like in individual droplets and if the organisms are metabolically active.
Methods: To obtain undisturbed water droplets, oil samples from the Pitch Lake (stored in jam jars under nitrogen atmosphere) were heated to ~45 °C. At warmer temperatures, the water droplets (lower density than oil) start to rise through the surface layer of the oil where the water can be picked up by pipettes. The droplets were solved in sterile particle free water stained with a final concentration of 5 µg ml^{-1} DAPI for 20 min and subsequently filtered through a membrane filter and counted via fluorescence microscope. The adenosine triphosphate (ATP) measurement was performed using a BacTiter-Glo™ Microbial Cell Viability Assay accordingly to the producer"s instructions. The droplets were diluted in sterile particle free water and 100 µl of Glo™ Reagent were added, afterwards the mixture was incubated for 5 min at room temperature before the samples were measured via luminometer.
Results: More than 50 % of the water droplets contained small microbial communities, with an average of 32,000 up to 40,000 cells $µl^{-1}$. ATP measurements of further droplets showed that 50 % of these contained an average of $4.7*10^{-15}$ mol ATP $µl^{-1}$, resulting in a mean value of $1.4*10^{-19}$ mol ATP per cell. This is compared to other active environmental samples a typical value. Furthermore, the activity has been confirmed by light microscopic investigations in which motile cells were visible.
Conclusion: Not all droplets enclosed in the oil contain microorganisms, but both methods combined show that these tiny water droplets in the oil are the habitat for ATP containing, active microbial communities.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

367/EEP
Thermophilic bacteria isolated from different environments and their ability to cellulose degradation
L. Lipinska*[1,2], M. Leszczewicz[1,2], K. Makowski[2,3], I. Kijewska[2], R. Wilczek[2], P. Komorowski[2,3], B. Walkowiak[2,3]
[1]*Politechnika Lodzka (Lodz University of Technology), Institute of Fermentation Technology and Microbiology, Lodz, Poland*
[2]*Lodz Regional Science and Technology Park Ltd., Lodz, Poland*
[3]*Politechnika Lodzka (Lodz University of Technology), Institute of Materials Science and Engineering, Lodz, Poland*

Introduction: Cellulose is a natural polymer and important structural component of the primary cell wall of plants, algae and the oomycetes. Process of cellulose degradation to glucose and dissolvable sugars is catalyzed by cellulases, which can be obtained from some bacteria and fungi. Thermophilic, cellulose-degradable bacteria, play an important role in bioconversion of cellulose to value-added bioproducts.
Objective: The aims of the study were an isolation of thermophilic bacteria from different environments, selection of cellulose-degrading strains and determination of their cellulolytic potential.
Materials & methods: Forty strains of thermophilic bacteria have been isolated from different environments in Poland (hay) and Island (karst spring, geothermal pont, geyser). They were tested toward the ability of growth in range of temperature between 50°C ÷ 80°C. We have determined the optimal temperatures and growth curves for every strain using Microbioreactor System BioLector® (m2p-labs, Germany). Thereafter, we have selected thermophilic strains capable to degradation of cellulose. Bacteria were cultivated under optimal conditions on agar plates with carboxymethylcellulose (CMC) as a sole carbon source. Strains able to degradation cellulose degradation were propagated in media with beet pulp, which is by-product from the processing of sugar beet. Products of cellulose degradation were characterized using Capillary electrophoresis (CE) with PA 800 plus system (CEofix carbo kit, Beckman Coulter).
Results: We have isolated thermophilic bacteria demonstrating diversified optimal temperatures of growth and morphology. The strains with cellulase activity were chosen, and their products of degradation of cellulose were identified and quantified using capillary electrophoresis. We have observed significant differentiation of enzymatic abilities and of production of glucose and other soluble sugars by tested thermophilic bacteria.
Conclusion: Selected thermophilic strains show activity of cellulases. They can find widespread application in bioconversion of waste from food, agro, and wood industries.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

368/EEP
Effects of soil and organic matter stratification on soil microbiomes and their potential to produce exo- and lipopolysaccharides
B. Cania*[1], M. Krauss[2], A. Fliessbach[2], M. Schloter[1], S. Schulz[1]
[1]*Helmholtz Zentrum München, Research Unit Environmental Genomics, Neuherberg, Germany*
[2]*Research Institute of Organic Agriculture, Department of Soil Sciences, Frick, Switzerland*

Conservation tillage compared to conventional tillage induces the accumulation of organic matter in the top soil layers and improves aggregate formation. This in turn supports plant growth and reduces soil erosion. Microorganisms play a crucial role in soil aggregation by the production of exopolysaccharides (EPS) and lipopolysaccharides (LPS) acting as glue for soil particles. We postulate that intensive tillage disrupts microbial communities, and thus affects EPS/LPS production. To test this hypothesis, we sampled soil layers from 0-10 cm, 10-20 cm, and 20-50 cm of ploughed and reduced tilled plots from a long-term field trial in Frick (Switzerland). The sampling took place in March 2015, before tillage and the subsequent maize cropping. To identify potential differences in microbial taxonomy and functionality, we extracted DNA from the samples using a phenol-chloroform based DNA/RNA extraction method, prepared metagenomic libraries with the NEBNext® Ultra™ DNA Library Prep Kit and subsequently performed shotgun sequencing on a MiSeq® sequencer. Taxonomic analysis of the metagenomic data revealed a distinct spatial influence on the composition of soil microbiomes, but only few differences between the tillage treatments. The observed differences in soil depth were mainly explained by shifts in the abundance of the dominant bacterial families, i.e. *Bradyrhizobiaceae*, *Chitinophagaceae* and *Cytophagaceae* were more abundant in 0-20 cm, while *Anaerolineaceae* and *Nitrospiraceae* had higher abundance in 20-50 cm. Functional analysis focused on genes catalyzing the biosynthesis and export of alginate, colanic acid, levan and other extracellular and capsular polysaccharides, as well as lipopolysaccharides. Depth was again the main factor affecting the distribution pattern of the analyzed genes. Specifically, the relative abundance of genes wcaF and lptFG encoding

respectively for a colanic acid acetyltransferase and permeases of the LptBFGC LPS export complex decreased with depth. Overall, genes encoding for the LptBFGC complex and a polysaccharide export outer membrane protein Wza were most abundant. All dominant bacterial families, except *Anaerolineaceae* and *Polyangiaceae,* harbored them. *Anaerolineaceae* had a very small potential to produce EPS and LPS, while *Polyangiaceae* showed no potential to synthesize LPS. Families that harbored most genes related to EPS/LPS production were *Chitinophagaceae*, *Nitrospiraceae* and *Planctomycetaceae*. As for taxonomic diversity, the two tillage forms had only a minor influence and induced mainly shifts in the microbial groups harboring the related genes. Obviously, long-term tillage does not affect microbial community composition unlike short-term influences reported in other studies. Consequently, future research needs to address more transcriptomes to investigate how different forms of tillage trigger gene expression.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

369/EEP

Magnitude and molecular characterization of multidrug-resistant gram-negative bacteria from inanimate surfaces of a Tanzanian tertiary hospital environment

N. Moremi*[1,2], H. Claus[1], V. Silago[2], P. Kabage[2], U. Vogel[1], S. E. Mshana[2]
[1]*Institut für Hygiene und Mikrobiologie, Wuerzburg, Germany*
[2]*Catholic University of Health and Allied Sciences, Mwanza, Tanzania*

Background: Contamination of inanimate hospital environment has been linked to several outbreaks of healthcare-associated infections caused by multidrug-resistant gram-negative (MDR-GN) bacteria.
Objective: To assess the magnitude of hospital surfaces contamination by MDR-GN bacteria in the Bugando Medical Centre (BMC).
Methods: A total of 138 non-repetitive hospital surfaces swabs were collected between June and August 2015. Samples were cultured onto MacConkey Agar. Extended spectrum beta lactamase (ESBL) screening was done using MacConkey Agar supplemented with 2µg/ml of cefotaxime. Identification and antimicrobial susceptibility testing were done by VITEK-MS and VITEK-2 system respectively. Genotyping was done using multilocus sequence typing. Data were analyzed using STATA-13 software.
Results: Of 138 BMC hospital surfaces swabs, 48 (34.7%) had significant growth of gram-negative bacteria. Isolated bacteria were *Enterobacter cloacae* 18 (37.5%), *Acinetobacter baumannii* 13 (27.1%), *Klebsiella pneumoniae* 12 (25%) and *Escherichia coli* 5 (10.4%). All 48 isolates were resistant to at least 3 classes of antimicrobials, 34 (70.8%) were ESBL-producing Enterobacteriaceae and one *A. baumanii* was resistant to carbapenems. *E. coli* sequence type (ST) 405 was found to circulate in different BMC wards with an exception of the premature unit resuscitation bed which had *E. coli* ST410. *E. cloacae* ST513 colonized neonatal ICU weighing scale and adult ICU bed. *Bla*OXA-23 gene was detected in carbapenemase-producing *A. baumanii*.
Conclusion: Contamination rate of hospital surfaces with MDR-GN bacteria is alarmingly high. Detection of ESBL-producing *E. coli* strains having STs 405 and 410 that were previously detected in clinical isolates in the same hospital calls for urgent improvement of infection and prevention control practices in the different units at BMC hospital.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

370/EEP

Persistence of *Salmonella* in agricultural soil

J. Schierstaedt*[1], S. Jechalke[2], E. Fornefeld[3], R. Grosch[1], K. Smalla[3], A. Schikora[3]
[1]*Leibniz Institute of Vegetable and Ornamental Crops, Department Plant Health, Großbeeren, Germany*
[2]*Justus Liebig University, Institute for Phytopathology, Giessen, Germany*
[3]*Julius Kühn-Institute, Institute for epidemiology and Pathogen Diagnostics, Braunschweig, Germany*

In the last years, salmonellosis outbreaks were increasingly associated with contaminated fruits and vegetables. This indicates that plants are suitable hosts for *Salmonella enterica*. Contamination of produce can occur along the whole production chain also, for instance, during plant growth. The survival of *Salmonella* in soil is an essential precondition for the colonization of plants. However, so far the knowledge about factors influencing its persistence in soil and in plant environment is scarce, and the question whether *Salmonella* uses plants as opportunistic bacterium or if it behaves as a plant pathogen is still controversially discussed.
We analyzed the influence of soil fertilization and soil sterilization on the survival of *Salmonella*. We observed an adaption of *Salmonella* inoculated into soil with reduced diversity that leads to enhanced persistence and survival in the plant environment. While fertilization with pig manure had a positive effect on the survival of *Salmonella* in soil, chicken manure had no distinct influence on the survival. Usually, sterilization of soil by autoclaving does not lead to a sterile soil but to a drastic reduction of the abundance and diversity of soil bacteria. *Salmonella* was able to survive in this soil for a relatively long time (monitored up to 6 months) and seemed to adapt to this environment. This adaption led to a change in the persistence in the presence of plants. Despite an initial decline, our data indicated a long-term survival of *Salmonella* in agricultural soil. The presence of the indigenous soil microbial community reduced its survival, most likely due to competition for resources.
Together, our results indicate that *Salmonella* can persist in soil for extended times and that adaptation to the soil environment enhances the risk of contamination of produce in the agricultural environment. The fact that *Salmonella* uses plants as alternative hosts strongly suggests that plants represent a much larger reservoir for animal pathogens than estimated so far.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

371/EEP

A prominent spring bloom forming member of the class Flavobacteriia represents a novel candidate genus closely related to *Ulvibacter*

T. Francis*[1], K. Krüger[1], B. Fuchs[1], R. Amann[1]
[1]*Max Planck Institute for Marine Microbiology, Molecular Ecology, Bremen, Germany*

Spring phytoplankton blooms in the North Sea are known to precipitate an ecological succession of heterotrophic bacterial clades, typically comprising members of the Bacteroidetes class Flavobacteriia, and Gammaproteobacteria, that is responsible for considerable remineralisation of algal biomass (Teeling et al., 2012; 2016).
One flavobacterial clade, previously considered a member of the genus *Ulvibacter*, blooms sooner than others following the initial rise in chlorophyll *a* during a bloom event. While it is known that other bloom forming bacteria make use of specific degradation pathways for complex polymers, the relatively rapid development of seemingly genetically homogeneous populations of this clade suggests it may make use of a specific labile substrate.
This study intended to gather phylogenetic and genomic information about the uncultured spring bloom forming clade

related to *Ulvibacter*, in order to examine what may permit the formation of such blooms.
Cells sampled from station Kabeltonne at Helgoland island in the North Sea were targeted for flow sorting using fluorescence in situ hybridisation. A mini-metagenome of these cells was then binned, and relationships between the resulting partial genomes were compared by average nucleotide identity. The genome fragments were analysed for the presence of polymer degrading genes, to assess potential substrate use.
A full-length 16S rRNA sequence of the sorted cells was also collected, and phylogenetic reconstruction using neighbour joining and maximum likelihood methods was used to establish the relationship of this clade to *Ulvibacter* and related genera. Pairwise similarity between 16S sequences was used to assess divergence between clades.
The bloom forming clade was found to consistently form a distinct cluster of potentially three species most closely related to the genus *Gilvibacter* (Khan et al., 2007). *Ulvibacter* type strains, and additional related genera, are clearly separated from this group. Pairwise similarity of <93.9% indicates that the bloom forming clade represents a novel genus distinct from *Gilvibacter*.
The sorted cell mini-metagenome contained two partial genomes, and ANI values showed these to be two species more closely related to each other than either one is to other *Ulvibacter* species.
Preliminary annotation of the metagenome bins does not support specialisation for polysaccharide utilisation.

References
[1] Teeling H et al. Science, 336:608-11, (2012)
[2] Teeling H et al. eLife 5:e11888, (2016)
[3] Khan ST et al. International journal of systematic and evolutionary microbiology 57(2):265-9, (2007)

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

372/EEP
Dining on iodine? How biofilm microbes cope with iodine.
L. A. Henning*[1], C. Karwautz[1], T. Lueders[1]
[1]*Institute of Groundwater Ecology, Helmholtz Zentrum München, Neuherberg, Germany*

The importance of iodine for human health and especially during fetal development is well established. Moreover, suspensions of molecular iodine are widely used as disinfectants. In contrast, not only the parameters of iodine ecotoxicology on microbes, but also possible redox transformations of microbes in biogeochemical iodine cycling have been less well investigated.
Here, we have isolated a number of microaerophilic bacteria from the massive biofilms recently discovered in a cave in southern Germany. This cavern of a former medicinal spring is rich in methane and iodine seeping from uprising deep formation water. The diversity of methano- and methylotrophs in the massive biofilms that completely cover the cave wall and ceiling have been previously reported here (Poster by Karwautz et al., VAAM 2016).
We now aim to investigate the adaption of the biofilm bacteria to the high iodine loads in the cavern water and the biofilms. Our initial work focusses on the biocidal effects of iodine and other iodine species (iodide [I^-], iodate [IO_3^-], iodomethane [CH_3I]) on a number of representative bacteria isolated from the biofilms. An ecotoxicity test for different iodine species is established via analysis of ATP, live-dead staining combined with FACS, and comparative growth curves. First tests show a decreasing growth and viability of Methylotrophs isolated from the biofilm under increasing concentrations of iodate.
Our next step now aims to isolate bacteria from the biofilms with a capacity of thriving on different iodine species either as electron donors or acceptors. Only a small diversity of iodide-oxidizing bacteria, e.g. distinct *Pseudomonas* spp., and also some microbes capable of iodate reduction has been reported to date. A potential role of cavern biofilms in production and uptake of toxic volatiles such as iodomethane is also investigated. In summary, this ongoing work will allow for a more comprehensive perspective of microbial iodine cycling in a unique geomicrobiological habitat.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

373/EEP
Visualization of microbial life on marine sand grains
D. Probandt*[1], T. Eickhorst[2], R. Amann[1], K. Knittel[1]
[1]*MPI Bremen, Molecular Ecology, Bremen, Germany*
[2]*University of Bremen, Soil Microbial Ecology, Bremen, Germany*

Sediments at the continental shelf are mainly permeable sands that are flushed with bottom waters containing organic matter and oxygen (1). More than 99% of the benthic community lives attached to sand grains (2), thus exposed to mechanical shearing and constantly changing environmental conditions. Bulk extraction of nucleic acids from several mg of sediment and subsequent 16S rRNA gene tag sequencing has revealed diverse microbial communities. In situ quantification of dislodged cells using fluorescence in situ hybridization (FISH) showed the presence of metabolically diverse clades including aerobic as well as anaerobic microbes (3). However, none of these methods allowed the analysis of the distribution and localization of the microbes directly in their natural environment, i.e. attached to sand grains.
In this study, we established workflows for i) micro computed tomography (micro-CT) of permeable sediments,for ii) CARD-FISH of microbial communities living on single native sand grains using confocal laser scanning microscopy and for iii) single sand grain-PCR to study the microbial diversity on exactly one sand grain. Micro-CT reconstruction of the top 2 cm of permeable sediments sampled in the southern North Sea revealed a well-connected pore space and related solid-pore interfaces enabling an efficient supply of the benthic community with solutes from bottom waters. Sand grains were colonized by microbial cells of diverse morphologies and microalgae as visualized by SYBR green I staining (~0.1-30% of total surface). Epi-growth was dominantly found in cracks and fissures well protected from mechanical abrasion. Using specific probes for CARD-FISH, we detected *Desulfobacteraceae*, *Planctomycetaceae* and *Acidobacteria* as well as *Phycisphaerae*, *Archaea* and microalgae living in close physical association on a single sand grains. Our results indicate that a major fraction of the microbial community on individual sand grains is adapted to present environmental conditions likely depending on spatial and seasonal availability of electron donors and acceptors. By that the benthic microbial community can react quickly and is thus particularly efficient in the remineralization of organic carbon in surface sediments. Amplification of 16S rRNA genes from a single sand grain was successful. Detected microbial diversity will be discussed in the context of CARD-FISH results and compared to the diversity of the (meta-)community retrieved from pooled sand grains.

References
[1] Huettel, M. *et al*., *Annual Review of Marine Science* (2014) 23-51.
[2] Rusch, A. *et al*., *FEMS Microbiol Ecol* (2003) 44: 89-100
[3] Musat, N. *et al*., *Syst Appl Microbiol* (2006) 29: 333-348

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

374/EEP
Influence of nitriles on bacterial communities
R. Egelkamp*[1], T. Zimmermann[1], R. Hertel[1], R. Daniel[1]
[1]*Georg-August-University Göttingen, Genomic & Applied Microbiology, Göttingen, Germany*

Nitriles are an important group of molecules for industry, especially as pharmaceuticals with a broad spectrum from antidiabetic drugs to standards in cancer treatment. A major problem dealing with nitriles is their cytotoxicity and enrichment of nitriles in natural habitats could lead to ecological problems. As nitriles can also occur as natural compounds, for example as plant defense agents, detoxification can occur by degradation pathway involving nitrilases, which degrade nitriles to carboxylic acids and ammonia. Despite the knowledge on microbial degradation pathways of nitriles, the influence of nitriles on composition of bacterial communities has not been studied. For this purpose, liquid cultures inoculated with an environmental sample were treated with ten different nitriles. Subsequently, growth and composition of the treated communities were monitored. Four of the ten tested nitriles are highly toxic to bacterial communities, as no growth was visible even after a long period of incubation. Treatment with the six remaining nitriles led to growth, which was in some cases better than the control group without nitriles. This indicated active nitrile degradation by microorganisms, giving them an additional nitrogen and carbon source. The 16S rRNA gene-based analyses of community composition of the six growing cultures revealed different and complex community structures, including several phylogenetic groups, which showed resistance against the influence of the nitriles.
To test for the active degradation of a nitrile via nitrilases, the same environmental starter community was incubated with acetonitrile as sole nitrogen source. Eight different microorganisms were isolated from the corresponding culture. Genome sequencing and analysis of the recovered isolates revealed the presence of genes encoding nitrilases and other genes potentially involved in nitrile utilization. Thus, this project contributes not only to the understanding of the influence of nitriles on bacterial communities, but also to identification of new biocatalysts for the green chemistry.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

375/EEP
Microbial hotspots revoke subsoil effects on microbial community structure and function
M. Fogt*[1,2], M. Uksa[3], D. Fischer[4], T. Kautz[5], M. Schloter[1,2], S. Schulz[1]
[1]*Helmholtz Zentrum München, Research Unit Environmental Genomics, Neuherberg, Germany*
[2]*Technische Universität München, Chair of Soil Science, Freising, Germany*
[3]*University of Hohenheim, Institute of Soil Science and Land Evaluation, Stuttgart, Germany*
[4]*Brazilian Agricultural Research Corporation - Embrapa, Seropédica, Brazil*
[5]*University of Bonn, Institute of Organic Agriculture, Bonn, Germany*

Previous studies clearly indicate that subsoils are soil layers depleted from oxygen. Therefore, microbial respiration coupled with organic matter degradation mainly in the rhizosphere and drilosphere, where easily degradable carbon is available is strongly influenced by the availability of electron acceptors. In this study we compared reconstructed redox processes for subsoil compartments including bulk soil, rhizosphere and drilosphere. We compared our data to bulk soil samples from the same compartments.
To investigate the microbial community composition as well as its functional potential for denitrification, sulfate reduction and methanogenesis we took soil samples from the rhizosphere and drilosphere as well as from bulk soil from the top and subsoil layer of an agricultural field in Klein-Altendorf, Germany. A taxonomic and functional analysis of microbial communities was based on a whole-genome shotgun sequencing of directly extracted DNA using Illumina MiSeq® technology. Library preparation was performed by applying NexteraXT Kit® from Illumina. The sequence reads were blasted against NCBI and KEGG databases for taxonomic and functional gene assignment, respectively.
Results clearly indicate that differences in bacterial taxonomy and functional genes distribution were more related to soil compartments than soil depth. The analysis of relative gene abundances revealed that denitrification and dissimilatory nitrate reduction (DNRA) predominates in the investigated compartments while sulfate reduction and methanogenesis are of minor importance. Drilosphere is enriched in genes encoding for both, denitrification and dissimilatory nitrate reduction while in rhizosphere only DNRA appears to be significant, especially in subsoil. According to taxonomical assignment for denitrification sequence reads Nitrospiraceae is the primary denitrifier in all compartments which might indicate coupled nitrification-denitrification processes. In drilosphere, however, Planctomycetaceae, Nitrososphaeraceae and Intrasporangiaceae are the most abundant families driving denitrification, while in rhizosphere Nocardiaceae, Chitinophagaceae, and Planctomycetaceae prevail.
Based on comparative metagenomic analysis we conclude that nitrate reduction is dominant anaerobic respiration process in subsoil, however, different compartments potentially favor other types of nitrate reduction pathways.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

376/EEP
Dilution culture experiments as a tool to determine the mortality factors of bacterial clades in coastal waters off Helgoland
N. Heinzmann*[1], B. Fuchs[1], R. Amann[1]
[1]*Max-Planck-Institut Bremen, Molecular Ecology, Bremen, Germany*

While factors inducing phytoplankton blooms and succeeding bacterioplankton blooms are intensively studied and fairly well understood, the factors responsible for the subsequent decrease in microbial abundance are not well explored yet. In general there are two main ecological factors that influence the mortality rates of bacteria: substrate limitation is responsible for a bottom-up control, while top-down control is exerted by protists and viral lysis. The aim of this study was to identify the key mortality factors of bacterioplankton in the coastal waters of Helgoland by dilution cultures. We prepared three different dilution cultures with seawater from Helgoland and followed the incubations for 16 days. The incubations were prepared with a 1:100 dilution of (1) 0.2 µm filtered sea water, (2) 0.2 µm filtered sea water and Zobell media or (3) diluted with artificial sea water enriched with Zobell media. To determine the microbial dynamics subsamples were taken every 3 - 12 h. Fluorescence microscopy was used for total cell and virus like particles (VLP) counts. Fluorescence *in situ* hybridization (FISH) and 16S rRNA tag-sequencing allowed the identification and quantification of bacterial communities. Over the course of the experiments both bacteria and VLPs increased in abundance in all incubations by a factor of 100 – 1,000 fold. The VLP counts showed different patterns in each incubation and were all in the range of 7×10^6 - 3×10^7 VLPs*ml^{-1}. In all incubations *Gammaproteobacteria* were the most abundant bacterial clade. Eukaryotic grazers have been determined and quantified by FISH and will be reported. Our results demonstrate that the controlled environments of dilution cultures could serve as a valuable tool to disentangle the mortality factors of bacterial groups, which would otherwise be difficult to follow *in situ*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

377/EEP

Fluorescent strains of *Bacillus anthracis* for microscopic tracking of infections in amoeba and other protists

P. Braun*[1,2], C. Kümpel[1], C. Balzcun[3,4], P. Scheid[3,4], G. Grass[1]
[1]*Bundeswehr Institute of Microbiology, Munich, Germany*
[2]*Ludwig-Maximilians-Universität München, Microbiology, Munich, Germany*
[3]*Central Institute of the Bundeswehr , Laboratory of Medical Parasitology, Koblenz, Germany*
[4]*University of Koblenz-Landau, Department of Biology, Parasitology and Infection Biology Research Group, Koblenz, Germany*

Bacillus anthracis, the causative agent of the zoonotic disease anthrax, is disseminated after the demise of a host from the animal carcass as endospores that can survive in the environment for several decades. Yet, we still know very little about the ability of the bacteria to replicate in the environment without the host-animal. In previously reported experimental settings *B. anthracis* was shown to multiply in *Acanthamoeba castelanii* and even survived as a saprophyte in soil. Currently however, it is unclear to which extend the pathogen is utilizing other soil-dwelling amoebae or non-amoebal, free-living protists, respectively.
The objective of this study was to use microscopically traceable *B. anthracis* strains for investigating their ability to infect and proliferate within a variety of soil-dwelling protists, mostly amoebae.
Using *Escherichia coli*/*B. anthracis* shuttle plasmids we constructed a variety of genetically modified fluorescently labeled strains of *B. anthracis* (derived from risk group 2 strains Sterne, Pasteur or CDC1014, respectively) harboring none or only one of the pathogens two intrinsic virulence plasmids. Endospores of these recombinant strains were purified and used to infect *Acanthamobae* spp. or other protists such as *Colpidium* sp. or *Euplotes aediculatus*, respectively. Infections were followed using laser-scanning microscopy. For this, a chamber-slide-based cultivation system was developed in order to easily follow bacteria-amoebae interactions.
This work is part of a continuum of research aimed at detailing a soil-borne life-cycle of the notorious zoonotic pathogen outside its animal hosts. The tested amoeba actively ingested the offered endospores. Most of these endocytosed endospores did not germinate, however, a small fraction developed into vegetative cells inside their non-animal host. The few endospores that germinated outside the amoeba were readily taken up by the protists and digested. Unexpectedly, other non-amoebal protists such as *Colpidium* sp. or *Euplotes aediculatus* exhibited auto-fluorescence in the emission wavelengths of the fluorescent proteins used. Thus, infections could not be followed. Instead these microbes were challenged with anthrax lethal toxin (LTx). Both species were susceptible towards LTx as determined by substantial changes in cell morphology. Possibly, use of marker proteins with different excitation maxima in the future will ameliorate this challenge and open a new window the hidden life of *B. anthracis* in unexpected hosts.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

378/EEP

Sulfamethoxazole (SMX) assimilating microbial populations from pig farm soil revealed by DNA/protein stable isotope probing and metagenomic analysis

W. Ouyang*[1], J. Su[2], Y. Zhu[2], H. Richnow[1], L. Adrian[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Department of Isotope Biogeochemistry, Leipzig, Germany*
[2]*Institute of Urban Environment, Chinese Academy of Sciences, Xiamen, China*

Veterinary antibiotics are widely used to accelerate animal growth and to prevent infectious farm diseases. A large share of the applied substances is excreted and subsequently pollutes soil and surface waters around livestock farms; these residues then propagate the development of antibiotic resistance genes causing severe public health concerns. Previous studies mainly depended on cultivation to isolate and characterize such degraders, while the wider picture of antibiotic degrading populations is missing.
In our study, we traced the flow of labelled antibiotics within a microbial community by identifying incorporation of ^{13}C into community DNA and community protein extracts to obtain information about the organisms in pig farm soil that integrate carbons from the antibiotics into their cell material. We chose the extensively used sulfamethoxazole (SMX) as a model compound. Garden soil regularly irrigated by effluents of a pig farm in Fujian province, China was sampled and incubated with 20 µg/g $^{13}C_6$-SMX or $^{12}C_6$-SMX for one month. The increase of $^{13}CO_2$ concentrations in the incubating bottle headspace during incubation demonstrated mineralization of SMX. However, also ^{13}C-labelled DNA was detected after gradient ultracentrifugation. 16S rRNA gene Illumina sequencing from different buoyant densities indicated several operational taxonomic units (OTUs) as SMX assimilating organisms, mainly affiliated to the Intrasporangiaceae, Thermoleophilia, Norcardiodaceae, Xanthomonadales and Pseudonorcadiaceae. In a subsequent experiment, proteins from a $^{13}C_6$-SMX fed community were separated by gel electrophoresis and analyzed by mass spectrometry to identify ^{13}C-labeled proteins based on a metagenomic database. Labelling ratio (LR) and relative isotope abundance (RIA) indicated involvement in SMX-anabolism. We identified 212 labelled proteins with RIA around 20% and LR from 0-100% including hydrolases which could play an important role in the degradation of SMX. Among the 212 labelled proteins, 78 were taxonomically annotated with high BLAST scores of >200 and 90% of their phylogeny are consistent with active players involved in SMX degradation identified by DNA-SIP, Intrasporangiaceae were the most abundant degraders accounting for 59%.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

379/EEP

Heterologous production of long-chain rhamnolipids from *Burkholderia glumae* in *Pseudomonas putida*

A. Wittgens*[1], F. Weggenmann[2], M. Henkel[2], R. Hausmann[2], F. Rosenau[1]
[1]*Ulm University, Center for Peptide Pharmaceuticals, Ulm, Germany*
[2]*University of Hohenheim, Institute of Food Science and Biotechnology, Department of Bioprocess Engineering (150k), Stuttgart, Germany*

Rhamnolipids are biosurfactants with an enormous potential for industrial applications based on their advantages in ecological acceptance, low toxicity and sustainability in comparison to petrochemical-based surfactants. They can be used e.g. as detergents in laundry products, dish washing liquids or as emulsifiers in cosmetics and foods. Beside the best known rhamnolipid producer *Pseudomonas aeruginosa* they are also produced by bacteria from the genus *Burkholderia*. Rhamnolipids are composed of either one (mono-rhamnolipids) or two rhamnose molecules (di-rhamnolipids) linked to one or mostly two 3-

hydroxyfatty acids with various chain lengths. In *P. aerugionsa* the length of the fatty acid chains in the rhamnolipids typically varies from C8 to C14 with a predominant species containing C10-C10 fatty acids. In contrast, rhamnolipids produced by bacteria from the genus *Burkholderia* contain long-chain fatty acids with length from C12 to C16 and a predominant C14-C14 species, which offer novel properties for potential industrial applications. The first reaction in rhamnolipid biosynthesis is catalyzed by RhlA, which is responsible for the synthesis of the fatty acid dimer 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs) as rhamnolipid precursor. Mono-rhamnolipids are synthesized by RhlB, which links dTDP-L-rhamnose to the HAAs. RhlC synthesizes di-rhamnolipids by adding a second rhamnose molecule to the mono-rhamnolipids. In contrast to *P. aerugionsa* all genes responsible for rhamnolipid biosynthesis are organized within a single gene cluster in rhamnolipid producing *Burkholderia* species.
We here established the biosynthesis of long-chain mono- and di-rhamnolipids by expressing relevant genes from *Burkholderia glumae* in form of biosynthetic *rhlAB* or *rhlABC* operons in the non-pathogenic *Pseudomonas putida* KT2440. *P. putida* is known as suitable host for heterologous rhamnolipid production and to circumvent the complex *quorum sensing* system, which natively regulates the rhamnolipid biosynthesis. We show that recombinant *P. putida* strains are able to produce the same rhamnolipid species containing long-chain fatty acids like the *B. glumae* wild type. Furthermore, we created biosynthetic operons containing hybrids of *rhlA* and *rhlB* genes from *B. glumae* and *P. aeruginosa* to demonstrate that RhlA determines the length of fatty acids used for rhamnolipid biosynthesis depending on its origin and not on the availability of predominant fatty acids in the host organism.
We could show that *P. putida* can produce different rhamnolipid species with various chain lengths, what makes him the ideal host to enlarge the portfolio of designer rhamnolipids for potential industrial applications.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

380/EEP

Physical and material properties of *Bacillus subtilis* biofilms

S. Kesel[1], S. Grumbein[2], A. Götz[1], C. Falcon Garcia[2], O. Lieleg[2], M. Opitz*[1]
[1]*LMU, Fakultät Physik, München, Germany*
[2]*IMETUM/Technische Universität München, Garching, Germany*

Many bacteria are able to embed themselves in a self-produced matrix, the so called biofilm. The biofilm thereby protects bacteria from antibiotics, chemicals and mechanical stresses, rendering the biofilm unremovable, which poses a big problem in health care and industry. In order to prevent the formation of biofilms or to cure patients from mature biofilms, it is necessary to understand which matrix component is responsible for the observed resistances and at which state of biofilm formation these resistances are developed.
Using different techniques1,2,3, such as e.g. cantilever arrays, time-lapse microscopy and atomic force microscopy (AFM), we investigated physical and material properties of the *Bacillus subtilis* biofilms NCIB 3610 and B-14 at different phases of biofilm formation, beginning with the attachment of single colonies to surfaces up to micro-colony growth and fully matured biofilms. To address the impact of specific biofilm matrix elements for the observed biofilm properties several mutant strains have been analyzed.

References

[1] S. Kesel, S. Grumbein, I. Gümperlein, M. Tallawi, A-K. M, O. Lieleg and M. Opitz. Direct comparison of physical properties of *Bacillus subtilis* NCIB 3610 and B-1 biofilms. Applied and Environmental Microbiology, 82(8): 2424-2432 (2016)
[2] S. Kesel, A. Mader, P.H. Seeberger, O. Lieleg and M. Opitz. Carbohydrate-coating reduces adhesion of biofilm forming *Bacillus subtilis* to gold surfaces**.** Applied and Environmental Microbiology, 80(19):5911 (2014)
[3] S. Grumbein, M. Opitz and O. Lieleg. Selected metal ions protect *Bacillus subtilis* biofilms from erosion. Metallomics, 6(8):1441-50 (2014)
[4] S. Kesel, F. Moormann, I. Gümperlein, A. Mader, M. Morikawa, O. Lieleg and M. Opitz. Draft Genome sequence of the biofilm producing *Bacillus subtilis* strain B-1 isolated from an oil field. Genome Announcements, 2(6): e01163-14 (2014)

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

381/EEP

Latex Clearing protein of *Streptomyces* sp. K30 (Lcp_{K30}) is a globin and employs Lysine 167 as an unusual distal haem ligand

W. D. Röther*[1], L. Ilcu[2], O. Einsle[2], D. Jendrossek[1]
[1]*University of Stuttgart, Institute of Microbiology, Stuttgart, Germany*
[2]*Albert-Ludwigs-Universität, Institute for Biochemistry, Freiburg, Germany*

Natural rubber is an item of daily use and vast amounts of waste, especially from tires, are permanently released into the environment. Research on its microbial degradation was significantly increased recently. Poly(*cis*-1,4-isoprene) represents the main component of the biopolymer and Gram-positives secrete Lcp that catalyzes its oxidation yielding oligo-isoprenoids (1). These can be employed as precursors for the production of fine chemicals or as biofuel. Lcps contain a haem *b* co-factor that is responsible for their red color (2). The sequences of all biochemically characterized Lcps include a domain of unknown function (DUF2236) enclosing only three highly conserved residues. Mutagenesis and biochemical characterization of purified muteins revealed that R164A and T168A were red, showed good protein stability, but were inactive indicating that both residues are essential for catalytic activity. H198A was also inactive and stable but was colorless due to the absence of haem. The co-factor of most haem-dependent proteins is coordinated by at least one and often by a second (conserved) histidine residue that function as axial porphyrin ligand(s). However, identification of a presumed second axial haem ligand was impossible (3).
We recently solved the 3D-structure of Lcp_{K30} (Fig.1) featuring a typical 3/3 globin fold as observed for e.g. myoglobin (4). The helices A-H are conserved forming a globin core capped by an additional *C*-terminal (magenta) and *N*-terminal domain (yellow). Notably helix D is missing whereas a short additional helix between F and G is present containing residue W211 that is conserved among Lcp homologues and therefore named L-helix (blue). We further confirmed residue H198 as the proximal haem ligand and located R164 and T168 in close proximity to the active site haem group (Fig.2) explaining their importance for the reaction mechanism. Surprisingly, the haem is additionally coordinated by Lysine 167 representing a highly unusual haem ligand.

References

[1] Watcharakul S, Röther W, Birke J, Umsakul K, Hodgson B, Jendrossek D. 2016. Biochemical and spectroscopic characterization of purified Latex clearing protein (Lcp) from newly isolated rubber degrading Rhodococcus rhodochrous strain RPK1 reveals novel properties of Lcp. BMC microbiology 16:92.
[2] Birke J**,** Röther W**,** Jendrossek D. 2015. Latex clearing protein (Lcp) of *Streptomyces* sp. strain K30 is a *b*-type cytochrome and

differs from rubber oxygenase A (RoxA) in its biophysical properties. Appl Environ Microbiol 81:3793–99.
[3] Röther W, Austen S, Birke J, Jendrossek D. 2016. Cleavage of Rubber by the Latex Clearing Protein (Lcp) of *Streptomyces* sp. Strain K30: Molecular Insights. Appl Environ Microbiol **82**:6593–6602.
[4] Bashford D, Chothia C, Lesk A. 1987. Determinants of a Protein Fold –Unique Features of the Globin Amino-Acid-Sequences. J Mol Biol 196:199-216.

Figure 1: 3D-Structure of Lcp_{K30} and myoglobin in a 3-3 globin fold:

Figure 1

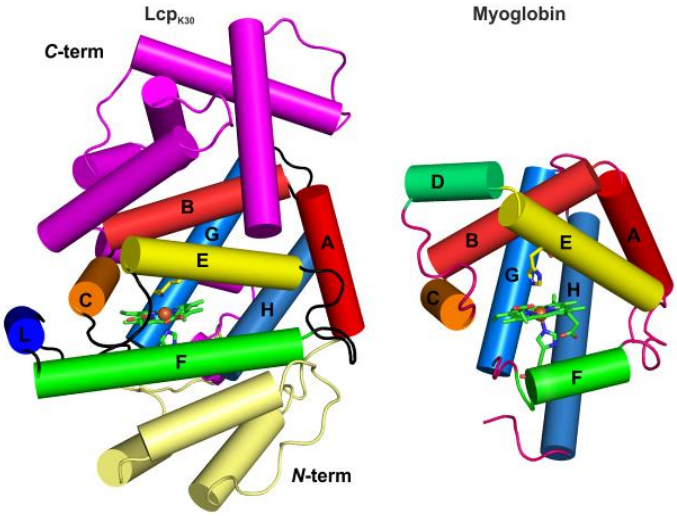
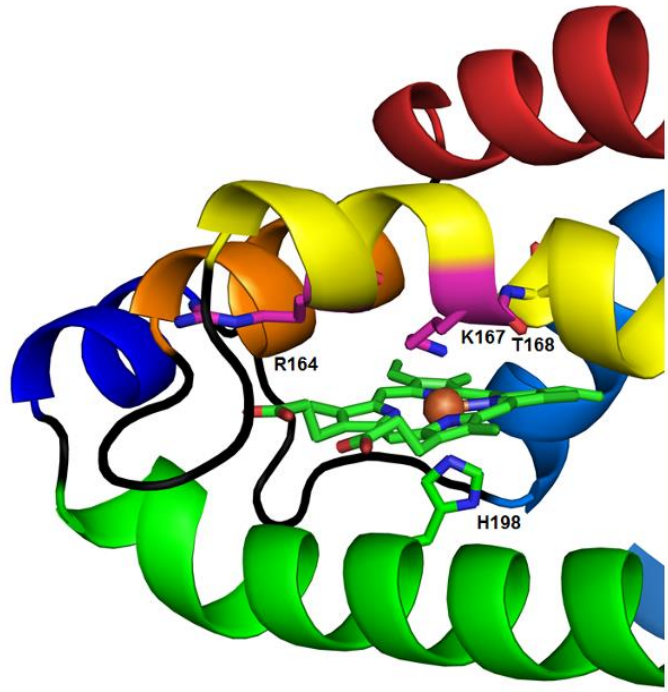


Figure 2: Active site of Lcp_{K30}:
Figure 2



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

382/EEP

Translating physics to microbiology: spore resistance to terrestrial and extraterrestrial extremes

R. Moeller*[1]
[1]*German Aerospace Center, Space Microbiology Research Group, Cologne, Germany*

Spore-forming bacteria are of particular concern in the context of planetary protection because their tough endospores are capable of withstanding certain sterilization procedures as well as harsh environments (Nagler et al., 2015, 2016; Nicholson et al., 2012). Spores of *Bacillus subtilis* have been shown to be suitable dosimeters for probing extreme terrestrial and extraterrestrial environmental conditions in astrobiological and environmental studies. During dormancy spores are metabolically inactive; thus substantial DNA, protein, tRNA and ribosome damage can accumulate while the spores are incapable of repairing and/or degrading damaged DNA and proteins. Consequently, damage to essential components of spores poses a unique problem, since damage repair does not occur until the processes of spore revival. Spores appear to have two possible ways to minimize deleterious effects of environmental extremes: (i) by protecting dormant spore macromolecules (in particular the spore DNA) from damage in the first place and (ii) by ensuring repair of damage during spore outgrowth. In our research, we used spores of different genotypes of *B. subtilis* to study the effects of various extraterrestrial conditions (e.g., planetary conditions as present on Mars or low Earth orbit (LEO)) for astrobiological purposes. Spores of wild-type and mutant *B. subtilis* strains lacking various structural components were exposed to simulated Martian atmospheric, galactic cosmic and UV irradiation conditions. Spore survival was strongly dependent on the functionality of all of the structural components, with small acid-soluble spore proteins, coat layers, and dipicolinic acid (DPA) as key protectants. In addition, the interaction of several DNA repair mechanisms (e.g., non-homologous end joining (NHEJ) and spore photoproduct (SP) lyase) was identified as crucial for surviving environmental extremes in space or Martian surface (i.e., exposure to solar UV and galactic cosmic radiation (Moeller et al., 2012). The ultimate goal is to obtain a complete model describing spore persistence and longevity in harsh environments.

References

[1] Moeller R, Schuerger AC, Reitz G, Nicholson WL. Protective role of spore structural components in determining *Bacillus subtilis* spore resistance to simulated mars surface conditions. Appl Environ Microbiol. 2012 Dec;78(24):8849-53. doi: 10.1128/AEM.02527-12.
[2] Nagler K, Julius C, Moeller R. Germination of Spores of Astrobiologically Relevant *Bacillus* Species in High-Salinity Environments. Astrobiology. 2016 Jul;16(7):500-12. doi: 10.1089/ast.2015.1419.
[3] Nagler K, Moeller R. Systematic investigation of germination responses of *Bacillus subtilis* spores in different high-salinity environments. FEMS Microbiol Ecol. 2015 May;91(5). pii: fiv023. doi: 10.1093/femsec/fiv023.
[4] Nicholson WL, Moeller R; PROTECT Team., Horneck G. Transcriptomic responses of germinating *Bacillus subtilis* spores exposed to 1.5 years of space and simulated martian conditions on the EXPOSE-E experiment PROTECT. Astrobiology. 2012 May;12(5):469-86. doi: 10.1089/ast.2011.0748.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

383/EEP

Methanogenic biodegradation of crude oil storage tank sludge enhances bio-corrosion of mild steel

C. Okoro*[1]
[1]*Federal University, Ndufu-Alike, Ikwo, Microbiology, Abakaliki, Nigeria*

Methods: Methanogenic biodegradation of crude oil sludge and corrosion of mild steel was investigated using chemical and molecular approaches.
Results: 16S rRNA gene sequences recovered from the samples revealed significant presence of Marinobacterium (63%), Pseudomonas (3%) alongside with acetotrophic Methanosaeta (16%) and hydrogenotrophic Methanobacterium (5%). The resident microbial community was able to reduce the gravimetric weight of residual oil by 65.5% (with complete degradation of C5-C17 nAlkane fractions) in non-amended samples and 94.13% (with complete degradation of C5-C25 nAlkane fractions) in substrate amended samples during the 60-day incubation period. As biodegradation progressed, acetotrophs consume acetate at the rate of 0.41Mm/day-1 while hydrogenotrophs consume hydrogen at the rate of 0.59Mm/day-1. Our results showed that the resident methanogenic archaea were largely responsible for the degradation of hydrocarbons in crude oil sludge and degradation rates were enhanced with substrate amendment which further accelerated the corrosion rates of mild steel coupons.
Conclusion: Considering the relatively high number of facultatively anaerobic marinobacterium and significant presence of *Pseudomona*s in the sequenced data, we speculate that the bacteria were at least partially responsible for biodegradation of crude oil components potentially acting as syntotrophic organisms with methanogens to convert crude oil to methane and enhance corrosion rates of mild steel.

References

[1] Andrade LL, Leite DC, Ferreira EM, Ferreira LQ, Paula GR, Maquine MJ, Hubert CR (2012). Microbial diversity and anaerobic hydrocarbon degradation potential of an oil contaminated mangrove sediment. BMC Microbiol. 12:186.
[2] Berdugo-Clavijo C, Gieg LM (2014). Conversion of crude oil into methane by a microbial consortium enriched from oil reservoir production waters. Front. Microbiol. 5: 197. Doi. 10.3389/fmicb.2014.00197.
[3] Fowler SJ, Toth CRA, Gieg LM (2016). Community structure in the methanogenic enrichments provides insight into syntrophic interactions in hydrocarbon impacted environments. Front. Microbiol. 7: 562. Doi. 10. 3389/fmicb.2016.00562.
[4] Gieg IM, Fowler SJ, Berdugo-clavijo C (2014). Syntrophic biodegradation of hydrocarbon contaminants. Curr. Opin. Biotechnol. 27: 21-29.
[5] Okoro C, Smith S, Chiejina L, Lumactud R, An D, Park HS, Voordouw J and Voordouw G. (2014). Comparison of microbial communities involved in souring and corrosion in offshore and onshore oil production facilities in Nigeria. J. Ind. MicrobioL Biotechnol. 41:665-678.
[6] Okoro, C.C.(2016). Microbial Communities of Light Crude from Nigeria and Potential for In-situ Biodegradation, Souring and Corrosion**.** Pet. Sci. Technol. 34(1):71-77.
[7] Okoro C C**,** Nwezza E E, Okpokwasili G C (2016)**.** Methanogen Population of Oil Production Skimmer Pit and the Effects of Environmental factors on Methanogenesis. Microb Ecol. 72(1): 175-184.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

384/EEP

Bentonite-geotechnical barrier and source for microbial life

N. Matschiavelli*[1], J. Steglich[1], S. Kluge[1], A. Cherkouk[1]
[1]*Helmholtz-Zentrum Dresden-Rossendorf, Biogeochemistry, Dresden, Germany*

The storage of highly radioactive waste is a challenging task for many scientists. For a deep geological deposition of the waste a multi-barrier concept is favoured, which combines a technical barrier (canister including the highly radioactive waste), a geotechnical barrier (e.g. Bentonite) and the geological barrier (host rock). Due to their properties, namely a high swelling capacity and a low hydraulic conductivity, Bentonites fulfil in this system a sealing and buffering function. Depending on the mineral composition Bentonites contain many suitable electron-donors and –acceptors, enabling potential microbial life. For the potential repository of nuclear waste the microbial mediated transformation of Bentonite could influence its properties as a barrier material. To elucidate the microbial potential within selected Bentonites, microcosms were set up containing a certain amount of Bentonite (20 g) and 40 ml anaerobic synthetic Opalinus-clay-pore water solution under an N_2/CO_2-gas-atmosphere. Substrates like acetate and lactate were supplemented to stimulate potential microbial activity. Microcosms were incubated in the dark, without shaking at 30°C. Within an indefinite time scale samples were taken at different time-points of incubation and were analysed regarding geochemical parameters like pH, O_2-concentration, redox potential, iron-concentration and sulphate-concentration as well as biological parameters like the consumption and formation of metabolites. First results show that bentonites represent a source for microbial life, demonstrated by the consumption of lactate and the formation of pyruvate. Furthermore, microbial iron-reduction was determined. The results reveal the importance of the selection of the best suitable Bentonite in order to avoid transformation by indigenous microbes.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

385/EEP

Impact of climate change on the diversity and abundance of bacteria living in the phyllosphere of a permanent grassland

S. P. Glaeser*[1], E. A. Aydogan[1], O. Budich[1], G. Moser[2], M. Hardt[3], C. Müller[2,4], P. Kämpfer[1]
[1]*Justus-Liebig-University Gießen, Department of Applied Microbiology, Gießen, Germany*
[2]*Justus-Liebig-University Gießen, Department of Plant Ecology, Gießen, Germany*
[3]*Justus-Liebig-University Gießen, Biomedical Research Centre Seltersberg-Imaging Unit, Gießen, Germany*
[4]*University College Dublin, Dublin, Ireland*

Global climate change leads to increasing atmospheric CO_2 concentrations and enhanced surface temperatures which can directly or indirectly affect the abundance and community structures of phyllosphere-inhabiting bacteria and in consequence plant-microbe-interactions.
The aim of this study was to get a first insight into the effects of elevated atmospheric CO_2 (eCO_2) and increased surface temperature (+2°C) on the composition and diversity of phyllosphere-inhabiting bacteria in a permanent grassland. Samples were collected at the Giessen Free Air Carbon Dioxide Enrichment (Gi-FACE) system which is continuously exposed to eCO_2 since more than 18 years and grassland exposed to increased surface temperature (+2°C) for 6 years. The localization of bacteria on the leaf surface was investigated by scanning electron microscopy and the total abundance of surface-attached bacteria was determined by Sybr Green I staining. A cultivation-dependent approach was applied for quantification and diversity study of

culturable bacteria focused on two abundant functional groups, heterotrophic bacteria and methylotrophic bacteria. Abundant bacteria (approx. 1000 isolates) were isolated and differentiated by 16S rRNA gene sequencing and genomic fingerprinting (BOX-PCR) up to the strain level. In parallel, total DNA was extracted from leaves and the phylogenetic composition of the phyllosphere inhabiting bacterial communities was compared by 16S rRNA gene amplicon sequencing using the Illumina technology.
Significant differences in the concentration and diversity of culturable heterotrophic and methylotrophic bacteria were found between plant species, CO_2 and surface temperature treatments. Similar changes were also obtained by the cultivation independent approach. *Sphingomonas* spp. represent one group of abundant phyllospheric bacteria affected in relative abundance and activity. A special *Sphingomonas* phylotype was only detected in the eCO_2 treatment and showed a specific substrate utilization pattern. Global climate change does not only lead to shifted microbial community compositions in the phyllosphere but also generates specific ecological niches for new bacterial phylotypes.
Results of this study are the first indication for an evolutionary adaptation of phyllosphere inhabiting bacteria induced by long-term exposure to elevated CO_2. Similar observations were also determined by long-term exposure to increased surface temperatures.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

386/EEP
Biodegradation of industrially polluted soil matrix
C. O. Esuola*[1,2,3], J. Trögl[2], M. Schlömann[1], O. O. Babalola[3], D. Tischler[1]
[1]*Technical University Bergakademie Freiberg, Institute of Biosciences, Environmental Microbiology, Freiberg, Germany*
[2]*Jan Evangelista Purkyně University, Department of Technical Sciences, Ústí nad Labem, Czech Republic*
[3]*North-West University, Food Security and Safety Niche Area, Mmabatho, South Africa*

Introduction: Toxic, heavy metals and hydrocarbons are still employed in many industries which anthropogenically leak into the environment. Unfortunately, the soil is the major sink of these toxic compounds which eventually pose as at health risk to life (humans, plants, animals and microbes). More research efforts are still needed to elucidate the role of bacteria for effective bioremediation of hydrocarbon and metal polluted soils.
Objectives: To investigate and determine the rate at which both *Gordonia* sp. and *Rhodococcus* sp. are effective for biodegradation of high concentrations of petroleum hydrocarbons in contaminated soil under the influence of metal-stress.
Materials & Methods: Contaminated soil (500 kg) were collected from an rafirenry lagoon and prepared for the pot experiment. A total of twelve variants, each in four replicates, was realized. 500g of soil was weighed into each pot (48 pots). The soil without addition of bacteria served as the control. Aliquot sampling were collected from each pot from 0-49 days. Samples were analysed for dehydrogenase activity, respiration activity, dry weight, aliphatic hydrocarbon (C10-C40) degradation and metal mobilization by ICP-MS. Data were calculated using confidence intervals (alpha=0,05) which allows pair comparisons of significance.
Results: Bioaugmentation, iron-stress and biocarrier (activated charcoal) enhanced dehydrogenase and respiration activity. The tested soil has a high concentration of Ca, Fe, Al, Mg. Other Trace elements, including As are also present at low concentrations. After 49 days, variant GR1; *Gordonia* sp. cultured in iron-contained bacteria salt media (BSM) added to soil, showed the highest degradation of the aliphatic hydrocarbons from ~80,000 mg/kg dry weight of soil to ~54,000 mg/kg.
Conclusion: *Gordonia* sp. and *Rhodococcus* sp. offer a biological degradation of industrially contaminated soil even under metal stress.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

387/EEP
Elucidation of key players and major metabolic routes of toluene degradation in a constructed wetland model system using molecular biological techniques and in situ protein SIP
H. J. Heipieper*[1], V. Lünsmann[1], U. Kappelmeyer[1], J. A. Müller[1], N. Jehmlich[2]
[1]*Helmholtz Centre for Environmental Research - UFZ, Department Environmental Biotechnology, Leipzig, Germany*
[2]*Helmholtz Centre for Environmental Research - UFZ, Department of Proteomics, Leipzig, Germany*

Constructed wetlands are well established for the treatment of wastewater and bioremediation of gasoline contaminated aquifers. The removal of organic compounds is carried out by microorganisms inhabiting the rhizosphere. Organic plant exudates and oxygen released by the roots stimulate the microbial degradation activity. These processes are called the rhizospheric effect. Despite the good performance of constructed wetlands, the understanding of the processes in the rhizosphere is still limited. In order to gain detailed knowledge about the process of organic pollutant degradation in the rhizosphere, we applied a model system, Planted Fixed-bed Reactor (PFR) which was running with toluene as sole external carbon source continuously for 6 years. In order to characterise the bacterial community and catabolic activities within this PFR, we applied DNA-based methods in combination with stable isotope fractionation and protein stable isotope probing (protein-SIP) techniques using 13C-toluene. In addition, abiotic parameters such as redox potential and oxygen concentration were monitored continuously. With these top notch approaches, the predominant pathway for toluene degradation as well as the bacterial key players carrying out this process could be identified.
The rhizospheric bacterial community was complex and numerically dominated by Rhizobiales and Burkholderiales, which contributed each about 20% to total taxon abundances. Notably, metaproteome and protein-SIP data revealed that *Burkholderiaceae* were main degraders of toluene in the wetland system. Thus, while the system was mostly hypoxic, toluene was preferentially degraded by aerobic processes. The initial degradation steps were apparently catalysed by *para* ring-hydroxylating monooxygenases which are known to be predominant under oxygen limiting conditions. A deeper insight into the molecular processes of toluene degradation was achieved by combining functional and taxonomic information. Our study is a promising proof of concept for future investigations of rhizospheric ecosystems degrading organic pollutants
Acknowledgements: This work was partially supported by a collaborative project (BACSIN, Contract No. 211684) of the European Commission within its Seventh Framework Programme.

References
[1] Lünsmann et al. 2016. *In-situ* protein SIP highlights *Burkholderiaceae* as key players degrading toluene by para ring hydroxylation in a constructed wetland model. Environ. Microbiol. 18:1176-1186.
[2] Lünsmann et al. 2016. Aerobic toluene degraders in the rhizosphere of a constructed wetland model show diurnal polyhydroxyalkanoate metabolism. Appl. Environ. Microbiol. 82:4126-4132.
[3] Martínez-Lavanchy et al. 2015. Microbial toluene removal in hypoxic model constructed wetlands occurs predominantly via the ring monooxygenation pathway. Appl. Environ. Microbiol. 81:6241-6252.

[4] Jehmlich et al. 2010. Protein-based stable isotope probing. Nat. Protocols 5:1957-1966.
[5] Stottmeister et al. 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnology Advances 22:93-117.
Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

388/EEP
Effect of rotor type and formamide concentration on the separation of isotope-labeled and unlabeled *Escherichia coli* RNA by isopycnic density ultracentrifugation
S. Weis*[1], E. Herrmann[1], C. U. Riedel[2], W. Young[3], S. Schnell[4], M. Egert[1]
[1]*Institute of Precision Medicine, Faculty of Medical & Life Sciences, Furtwangen University, Villingen-Schwenningen, Germany*
[2]*Institute of Microbiology and Biotechnology, University of Ulm, Ulm, Germany*
[3]*Grasslands Research Centre, AgResearch Ltd, Palmerston North, New Zealand*
[4]*Institute of Applied Microbiology, Research Center for BioSystems, Land Use, and Nutrition (IFZ), Justus–Liebig–University Giessen, Giessen, Germany*

Background: Separation of differentially isotope-labeled bacterial RNA by isopycnic density gradient centrifugation is a critical step in RNA-based stable isotope probing (RNA-SIP) analyses, which help to link the structure and function of complex microbial communities in their natural environments [1].
Methods & Results: Using ^{13}C isotope-labeled *Escherichia coli* RNA, we showed that an 8 mL near-vertical rotor performed better than a 2 mL fixed-angle rotor, thereby corroborating current recommendations [2, 3]. Neither increased concentrations of formamide nor urea in the medium improved the separation results using the fixed-angle rotor [3]. In follow-up experiments we showed that although a total omission of formamide during the ultracentrifugation resulted in a significant shift of RNA buoyant density towards lower values, the separation of isotope-labeled and unlabeled *Escherichia coli* RNA was not affected. In current RNA-SIP protocols [2] the addition of formamide as a denaturing agent is suggested to release RNA secondary structures, which might negatively affect the isopycnic separation of RNA during the ultracentrifugation step. However, formamide is also categorized as a CMR-reagent, i.e. as carcinogenic, mutagenic and toxic to reproduction.
Conclusion: Based on our findings, the addition of formamide might not be needed in RNA-SIP analyses. Clearly, an omission of formamide might increase the occupational safety of such analyses.

References
[1] Lueders T. et al. (2016). RNA-stable isotope probing: from carbon flow within key microbiota to targeted transcriptomes. Curr. Opin. Biotechnol. 41: 83-89
[2] Whiteley A.S. et al. (2007). RNA stable-isotope probing. Nat. Protoc. 2: 838-844
[3] Herrmann E. et al. (2016). Effect of rotor type on the separation of isotope-labeled and unlabeled *Escherichia coli* RNA by isopycnic density ultracentrifugation. Can. J. Microbiol. Published on the web 27 September 2016, 10.1139/cjm-2016-048.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

389/EEP
Glycerol fed biofilms derived from wastewater-electrochemical characterisation and microbial composition
S. Thiel*[1], B. Bunk[2], K. Huber[2], D. Jahn[1], J. Overmann[2], R. Biedendieck[1]
[1]*TU Braunschweig, Institute of Microbiology & BRICS, Braunschweig, Germany*
[2]*DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*

Introduction: In times of growing demand for clean and renewable energies bioelectrochemical systems take on greater significance. In microbial electrolysis cells (MEC) the chemical energy of wastewater gets converted to hydrogen and electricity. Undefined microbial electrochemically active biofilms are capable of oxidizing different electron donors, e.g. acetate, under anaerobic conditions and transferring released electrons to the anode. Anodic wastewater based biofilms are mostly dominated by Gram-positive *Geobacter* species that use direct extracellular electron transfer using cytochromes or/and nanowires to produce current.
Objectives: In an attempt to increase selection pressure, undefined primary biofilms were transferred to new electrochemical cells to generate a secondary electrochemically active biofilm. Hence, the microbial consortium among primary, secondary and tertiary biofilms should alter. Those pre-conditioned bacteria are capable of better biofilm formation, higher current densities and better coulombic efficiencies (CE). Additionally, our approach is the use of glycerol as electron donor as it is the main by-product of biodiesel production and can get converted to valuable platform chemicals for polymer industry.
Materials & methods: Electrochemical experimental setup: 500 mL half cell reactor, graphite electrodes, incubation at 35 °C, inoculum: domestic wastewater, electron donor concentration: 10 mM, applied potential: 0.2 V vs. Ag/AgCl. The microbial composition was analysed by partial 16S rRNA sequencing.
Results: Secondary and tertiary biofilms show more reproducible current profiles, higher maximal current densities and higher CEs compared to primary biofilms. Partial 16S rRNA sequencing revealed a *Geobacter sp*. domination of up to 60 % throughout primary, secondary and tertiary biofilms while negligible amounts of *Geobacter* sp. were found among planktonic cells.
It was shown that current production with glycerol as electron donor is possible. Maximal current densities of 0.69 mA/cm² and CE of up to 54 % with tertiary biofilms were obtained with a *Geobacter* sp. abundance of up to 45 % in the biofilm. The microbial consortium of glycerol fed planktonic cells was much more diverse than that with acetate as electron donor. Besides, a notable alteration of microbial consortium of pre-conditioned planktonic cells was revealed.
Conclusion: Increasing selection pressure led to more reproducible current profiles, higher current densities and better CE compared to primary biofilms. Acetate and glycerol fed biofilms were dominated by *Geobacter* sp. of up to 60 % and 45 %, respectively, while the microbial consortium of planktonic cells was more diverse with glycerol as electron donor. Future attempts will be focused on defined mixed cultures between *Geobacter sulfurreducens* and defined partners to establish a bioelectrochemical model system.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

390/EEP

Treatment of cellulose-containing substrates by micromycetes and anaerobic microbial communities.

L. Prokudina*[1], E. Tsavkelova[1], A. Netrusov[1]
[1]*Moscow State University, Microbiology, Moscow, Russian Federation*

In recent years, the amount of the biotechnological approaches in degradation of municipal wastes has greatly increased. One of the efficient processes of waste products recycling is the biotransformation of cellulose-containing materials (CCM) into biofuels, such as biogas, biohydrogen, biobutanol or bioethanol.
We studied the treatment of CCM by microorganisms in anaerobic and aerobic conditions. Different types of CCM were used: ashless filters, magazine paper, newsprint and office paper with black and white printing, corrugated cardboard and the mixture of all types of papers, as well as phytomass of Jerusalem artichoke (stems and leaves of *Helianthus tuberosum* L.), which were preliminary dried and cut into pieces of 0.5 cm^2. Anaerobic treatment was performed with two methanogenic communities: one of the cattle manure (*i*) and another, selected from different natural and anthropogenic sources (*ii*). Two micromycetes, *Aspergillus terreus* and *Trichoderma viride*, were used for the aerobic CCM degradation, as well as for a pretreatment step of further CCM bioconversion into biogas by anaerobic communities. We showed that the office paper, cardboard and a mixture of papers were the most utilizable CCMs. Among the micromycetes, the endoglucanase and the total cellulase activities of *T. viride* were 2-3 times higher than those of *A. terreus*. The cultivation conditions (composition of the culture medium, supplementation with the co-substrate, seeding technique) and the conditions of the fungus biomass treatment for its subsequent bioconversion into biogas by anaerobic microbial communities were optimized. For *T. viride*, the maximal activity of cellulases was 0.80 U/mL on the office paper and 0.73 U/mL on the paper mixture. The pretreatment with *T. viride* increased the efficiency of the substrate hydrolysis and thus, the biogas production by the (*i*) microbial community cultivated on the lignin-containing paper mixture. The maximum values of the cumulative methane content (52.3 %) were achieved when the fungi were cultivated for 3 days. The increase of 1.5 times in cumulative methane yield by the (*ii)* microbial community was also observed under fungal pretreatment of the phytomass of *H. tuberosum*.
The composition of the selected microbial communities was studied by scanning electron microscopy and metagenomic analysis. Less amount and activity in the substrate decomposition has been observed on the newsprint paper and magazine paper, whereas the most abundant microbial populations were observed on the corrugated carton and the paper mixture, where the microbial cells were embedded within the extracellular matrix attached to the substrate. Among the dominant isolates were *Thermoanaerobacterium thermosaccharolyticym, Ruminiclostridium thermosuccinigenes, Tepidimicrobium ferriphilum, Ruminiclostridium cellulosi*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

391/EEP

Are the mysterious dryland "Fairy Circles" the result of microbial phytopathogenesis?

A. van der Walt*[1,2], O. Reva[2], R. M. Johnson[1], M. Seely[3,4], S. Pointing[5], D. Cowan[1], J. B. Ramond[1]
[1]*University of Pretoria, Centre for Microbial Ecology and Genomics, Pretoria, South Africa*
[2]*University of Pretoria, Centre for Bioinformatics and Computational Biology, Pretoria, South Africa*
[3]*University of Witwatersrand, School of Animal, Plant and Environmental Sciences, Johannesburg, South Africa*
[4]*Gobabeb Research and Training Centre , Walvis Bay, Namibia*
[5]*Auckland University of Technology, Institute for Applied Ecology New Zealand, Auckland, New Zealand*

Fairy Circles (FCs; Fig. 1) are enigmatic barren circular patches of soil, enclosed in a grass fringe, which are found in drylands. While a substantial number of hypotheses have been presented (e.g., vegetation self-organization, micro-faunal activity, abiotic gas seepage) to explain their origin, none are completely consistent with their properties or distribution. In this study, we tested the original hypothesis that Fairy Circles constitute isolated infections by microbial phytopathogens using a sequence based approach. Furthermore, for the first time, we compared FCs in the three locations where they have been described to date: Namib Desert dune and gravel plain FCs as well as Western Australian FCs. We performed 16S rRNA gene and ITS region amplicon sequencing and full shotgun metagenome sequencing on control-vegetated and barren FC centre soils. We consistently detected potential phytopathogens in FC centres, some of which were FC-specific.
In silico analysis of metabolic potential indicated adaptation to these extreme environments, including predicted genes assigned to pathways involved in temperature response, quorum sensing, oxidative stress and chemotaxis. Moreover, open reading frames supporting microbial secondary metabolism (carotene and terpenoid biosynthesis) and protein secretion systems (e.g., bacterial Type I, II and IV) were detected. Altogether, these results suggest a potential role of microbial taxa in the etiology and/or maintenance of FCs.

Figure 1

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

392/EEP

Effect of antibiotics in the environment: disturbances of plant-bacterial endophyte interactions in soft rush, *Juncus effusus*, after repeated exposure to sulfamethoxazole and trimethoprim

M. Arslan*[1], M. Santoni[1,2], A. Wiessner[1], T. Neu[3], J. A. Müller[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Environmental Biotechnology, Leipzig, Germany*
[2]*Sapienza - Università di Roma, Dipartimento di Chimica, Rome, Italy*
[3]*Helmholtz Centre for Environmental Research - UFZ, Department of River Ecology, Magdeburg, Germany*

Introduction: Organic micropollutants such as antibiotics are recent ecotoxicological challenges for various environmental systems including their associated human societies. Most previous studies reported on the effects of such compounds on model organisms; comparatively little is known about their impact on plants and in particular the important endophytic community.
Objective: To elucidate disturbances of plant-endophytes interactions after exposures to the antibiotics sulfamethaxazole (SMX) and trimethoprim (TMP).
Methodology: Soft rush (*Juncus effusus*), a common wetland plant, was repeatedly exposed to low aqueous concentrations of SMX and TMP. Plant status was evaluated based on evapotransporation rates and by visual inspection. Changes of endophytic communities in roots and shoots of exposed and unexposed plants were tracked via culture-dependent (MPN, isolation of bacterial strains) and culture-independent (qPCR, FISH) approaches.
Results: During exposure to SMX and TMP, evapotransporation of plants decreased. Evapotransporation recovered in the absence of the antibiotics, albeit to lower values than prior exposure. After several exposures plants became infested with insects, evapotransporation was almost zero, and plant tissue turned necrotic. The comparative analyses of endophytic communities showed that roots of post-exposed plants had statistically significant increases of Gammaproteobacteria, Firmicutes, and Actinobacteria. Some of the isolated strains are related to previously described phytopathogens. Endophytic fungi were not observed in this study. Shoots harbored lower endophyte abundances than roots, and there was no statistical difference between exposed and unexposed plants.
Conclusions: While the direct cause for the decline in plant status after exposure to SMX and TMP is yet uncertain, it appears that it was due to a lasting dysbiosis of the endophytic community in the roots, triggered by treatment with the antibiotics.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

393/EEP

First report of the Plant growth promoting *Stentrophomonas maltophilia* IAUK1047 isolated from rhizospheric soil of a field in Kerman, Iran

F. Rokhbakhsh Zamin*[1], N. Kazemi Pour[1], Z. Hadiyan[1]
[1]*Islamic Azad University, Kerman Branch, Microbiology, Kerman, Islamic Republic Of Iran*

Background and aim: Plant growth promoting bacteria are recently used for improving the growth and yield of agricultural crops. So, it is very critical to screen the effective isolates from rhizosphere of different plants. Rhizobacterial diversity is a major resource for these biotechnological processes. The aim of this study was to isolate and characterize Persian native rhizobacteria with excellent plant growth promoting traits.
Methods: Soil samples of maize were collected from the rhizosphere of an agricultural field located in Kerman district (south east of Iran). All samples were used for isolation of rhizobacterial strains by using enriched and differential media such as Thompson and Pikovaskia. Nitrogen free media such as Jensens, Burk"s and Ashby"s were used for selection of root-associated diazotrophic rhizobacteria. The other tests were performed as phosphate and zinc solubilization, phosphatase test, nitrate reduction, ammonia production as well as sensitivity to different antibiotics and resistance to the adverse environmental factors. In the next step, the best strain was selected according the results of above mentioned tests and identified by molecular methods (PCR). Finally, the surface-sterilized seeds of maize cultivar (single cross 704) were treated with selected bacterial isolate to evaluate its effectiveness on the growth of the test plant under pot conditions. All treatments were arranged in separate pots with non sterile soil and after 21 days, dry weight, shoot height and root length were determined.
Result: 36 different rhizobacteria were isolated from rhizospheric soil of maize. One strain out of 36 rhizobacteria was selected for further studies according to the good and attractive results obtained from different performed tests. This isolate was identified as *Stentrophomonas maltophilia* IAUK1047 on the basis of molecular tests. Obtained results revealed that this strain could fix atmospheric nitrogen, produce ammonia and phosphatase with phosphate solubilization efficiency (120). This strain was sensitive to most antibiotics and its resistance to the adverse environmental factors such as high salt concentration, pH and temperatures was observed as 6, 10 and 41°C, respectively. Pot experiment was evaluated by using seeds of maize cultivar and IAUK1047 strain. Root height, shoot length and dry weight had significant positive changes according ANOVA analysis.
Conclusion: Results of the present study revealed that *Stentrophomonas maltophilia* IAUK1047 isolated from rhizosphere of maize has interesting capabilities such as nitrogen fixation, ammonia production as well as phosphate solubilization which it is the first report in our area. So, the present data corroborate the hypotheses that this isolate has the potential to act as the plant growth promoting rhizobacterium and can help the growth of maize and may be the other crop plants particularly under environmental conditions, where phosphate or nitrogen sources are limited.
Keywords: *Stentrophomonas maltophilia* IAUK1047, plant growth promotion, nitrogen fixation, phosphate solubilization, pot experiment

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

394/EEP

Reduction of micro-organisms in combined sewer overflow by passage through vertical-flow constructed wetlands (retention soil filters)

C. Schreiber*[1], T. Kistemann[1], K. Tondera[2]
[1]*Institute for Hygiene and Public Health (IHPH), University of Bonn, GeoHealth Centre, Bonn, Germany*
[2]*Institute of Environmental Engineering, RWTH Aachen University, Aachen, Germany*

Introduction: Wastewater contains numerous pathogens from faecal contamination. Normally, it is treated in sewage treatment plants (STP) before entering the environment. In case of heavy rain events variable wastewater flows may overload the STP and is then directly discharged into the receiving river without any treatment as combined sewer overflow (CSO).
Objectives: The main focus of the EU Bathing Water Directive is set on the indicator bacteria *E. coli* and intestinal enterococci (IE). However, viruses may pose higher health risk than bacteria to humans using surface waters for recreational purposes [1, 2]. Therefore, we evaluated the reduction of somatic coliphages as viral indicator in addition to *E. coli* and IE in CSO by retention soil filters (RSF) at four test-scale sites and two full-scale sites. At the test scale sites we tried to evaluate the reduction of micro-organisms depending on preceding dry phases, operation and filter depth under defined conditions. These results were compared to reduction in full scale treatment under real-live conditions. All research (projects "Swist IV", "ReB-Op" and "ReSMo") has been funded by the Ministry for Climate Protection, Environment, Agriculture, Conservation and Consumer Protection of the State of North Rhine-Westphalia.
Materials & methods: Samples were taken as random samples in sterile bottles and cooled until processing within 24 h in the laboratory. Bacteria were analysed by culturing in liquid media and on agar plates according to EN ISO 7899-1, EN ISO 7899-2 (IE), ISO 9308-3 and EN ISO 9308-1 (*E. coli*), respectively. Detection of somatic coliphages followed EN ISO 10705-2.
Results: Preliminary results by the test-scale sites showed no clear correlation between dry phases and removal efficiency yet, though there were indications that the efficiency for *E. coli* seems to be at an optimum after 6-7 days without feeding. The median removal for the indicators investigated was approximately 1 $\log_{10}$. The removal increased at serial operation and is less for 50 cm filter depth then for 75 cm depth.
The full scale retention soil filters showed different reduction rates varying by site and parameters. One site reduced microorganisms within the range of the test scale sites, while the other one showed reduction rates up to median 3 $\log_{10}$ [3].
Conclusion: The results can help to redesign the wastewater system as required due to local circumstances and hygiene requirements for the discharging surface waters and therefore reduce human health risks.

References

[1] McBride GB, Stott R, Miller W, Bambic D, Wuertz S (2013), Water Research 47, 5282–5297.
[2] Timm C, Luther S, Jurzik L, Hamza IA, Kistemann T (2016), International Journal of Hygiene and Environmental Health http://dx.doi.org/10.1016/j.ijheh.2016.07.017.
[3] Christoffels E, Mertens FM, Kistemann T, Schreiber C (2014), Water Science and Technology 70: 1503-1509.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

395/EEP

Metagenomics and FISH-CLEM visualization of marine sponge-associated Chloroflexi

K. Bayer*[1], B. Slaby[1], M. T. Jahn[1], L. M. Silva[2], U. Hentschel[1]
[1]*GEOMAR-Helmholtzcentre for Ocean Research, Marine Microbiology, Kiel, Germany*
[2]*The University of New South Wales, Centre for Marine Bio-Innovation, Sydney, Australia*

Marine sponges harbor an exceptional microbial diversity with up > 10.000 different operational taxonomic units (OTUs) being associated with sponge individuals, the vast majority of which remains uncultivated. Members of the phylum *Chloroflexi* can dominate the microbiome of the high microbial abundance (HMA) sponges. In order to elucidate the diversity and function of Chloroflexi symbionts within marine sponges, we combined amplicon sequencing with metagenomics and single cell genomic analysis, as well as microscopy (FISH-CLEM). A total of five metagenomics bins and 13 single amplified genomes of the classes Caldilineae, Anaerolineae and SAR 202 group were recovered from the sponge metagenomes; the six most complete genomes were then analyzed in more detail. Besides shared genomic features with respect to carbon and nitrogen metabolism, carbon degradation, and respiration, the analyzed Anaerolineae genomes were depleted in the number of CRISPR and secondary metabolism related gene clusters compared to their cultivated relative *A. thermophila* and also compared to the other *Chloroflexi* symbionts. We posit that the Caldilineae and SAR 202 type symbionts are responsible for bacterial defenses, while the Anaerolineae may benefit from this protection. Furthermore, the newly established method FISH-CLEM was applied, to our knowledge for the first time, to visualize *Chloroflexi* symbionts within the sponge extracellular matrix at ultrastructural resolution. This study contributes to our understanding of the basis of marine sponge-microbe interactions.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

396/EEP

Ethanol production from Organic Municipal Solid Waste using a novel enriched complex microbial community.

P. Carrillo-Barragan*[1], N. Gray[1], J. Dolfing[1], P. Sallis[1]
[1]*Newcastle University, Civil Engineering and Geosciences, Newcastle upon Tyne, United Kingdom*

Lignocellulosic ethanol is a promising sustainable alternative to fossil transport fuels. Research has been focused on the modification of single microorganisms to hydrolyse lignocellulosic substrates while fermenting the products into EtOH. However, this approach faces instability, narrow operational conditions and is susceptible to inhibition by contaminants. We propose the assembly of a community from natural environments where lignocellulose degradation occurs as a suitable alternative for the fermentation of a complex lignocellulosic substrate such as the Organic fraction of Municipal Solid Waste (OMSW) with manipulation of the physical environment as a means to direct the metabolism towards EtOH production.
Methods: An OMSW analogue was fabricated with food, paper and cardboard waste and then acid/steam pre-treated. Compost piles, forest soil, rumen fluid, manure, and sludge from an anaerobic digester treating paper industry waste were sampled and used individually and in combination as inocula in closed microcosms cultured under initial anaerobic and aerobic conditions, with and without pH adjustment at 20°C. The fermentation products were then monitored using GC-FID. Bacterial DNA was extracted from samples taken at different time points and Ion torrent sequencing was used to sequence barcode

tagged 16Sr RNA amplicon libraries. Pipeline analysis was performed using Mothur and Qiime software.
Results: EtOH was produced by all the inocula with no significant difference between microcosms growing under initial aerobic and anaerobic conditions. EtOH was the major product in rumen and sludge inoculated systems, both generating their highest EtOH concentrations (~35mM EtOH) when growing at different pH values (7 and 5, respectively). Rumen composition changed from an evenly distributed rich community into a less rich one dominated by *Pseudomonas sp*., a negligible component in rumen core microbiome (Henderson *et al*., 2015); whereas sludge shifted from a community dominated by a *Pseudomonas* species into a less rich community with *Clostridium sp.* as the most abundant species. Microcosms inoculated with both inocula also generated EtOH as the major metabolic product under the range of pH 5 to 7. In this new community, *Pseudomonas* and *Clostridium* co-existed being both the two most abundant species.
Conclusion: A novel microbial community able to produce EtOH from OMSW at a broader range of pH values than the individual inocula was enriched. As none of the known most abundant species in rumen were enriched in the new community, the results suggest that it is possible to exploit less common metabolic capabilities from complex communities when changing the environmental conditions as pH. Forthcoming chemical and molecular biology analysis will allow the study of the stability, activity, diversity and function of this new community after repeated subculturing. Forming the basis for of a basic model to help understand and optimize EtOH production by manipulating environmental conditions.

References
[1] Henderson, G. et al. (2015) Sci. Rep. 5, 14567; doi: 10.1038/srep14567 (2015)

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

397/EEP
Loss of cortactin facilitates the invasion of intestinal epithelial cells by *Salmonella enterica* sv. Typhimurium
A. Felipe-López*[1], T. Estrada-García[1], M. Hensel[2], T. Stradal[3], K. Rottner[4], M. Schnoor[1]
[1]*CINVESTAV, IPN, Molecular Biomedicine, Mexico City, Mexico*
[2]*FB05 Biologie/Chemie Universität Osnabrück, Abt. Mikrobiologie, Osnabrück, Germany*
[3]*HZI für Infektionsforschung Braunschweig, Zellbiologie, Braunschweig, Germany*
[4]*Zoologisches Institut. TU Braunschweig, Molekulare Zellbiologie, Braunschweig, Germany*

During invasion of *Salmonella enterica* sv. Typhimurium, enterocytes lose microvilli and suffer from strong disorganization of the F-actin cytoskeleton and, as a consequence, damage of the epithelial barrier. The F-actin binding protein cortactin supports the epithelial barrier function of the intestine by controlling actin dynamics at *tight* and *adherens junctions*. Unsworth *et al.* previously implicated that the recruitment of cortactin to the invasion site of *Salmonella* in epithelioid cells coincided with ruffle formation. Here, we set to investigate the role of cortactin for epithelial barrier functions during *Salmonella* invasion.
Caco-2 C2BBe1 cells were transduced using a lentivirus to downregulate the expression of cortactin. Microscopy analysis revealed that cortactin-depleted C2BBe1 cells appeared bigger having enlarged nuclei. Furthermore, they did not establish strong epithelial barriers compared to cells expressing a scrambled shRNA (scr) or non-transduced cells. To evaluate the contribution of cortactin to *Salmonella* invasion, we infected cortactin-depleted C2BBe1 cells with the *Salmonella* WT-strain SL1344 and a non-invasive Δ*invG*-strain. The *Salmonella* WT-strain invaded cortactin-depleted C2BBe1 cells thrice more efficiently than the control C2BBe1-cells (scr or non-transducted), whereas the Δ*invG* strain infected neither the control nor cortactin-depleted cells. Confocal microscopy revealed retracted cells and severely damaged tight junctions in the absence of cortactin compared to control cells. Additionally, *Salmonella* ruffles were two times larger without cortactin. To examine the functions of cortactin *in vivo*, we infected streptomycin pre-treated WT C57BL/6 and *cortactin*$^{-/-}$ (*cttn*$^{-/-}$) mice with SL1344 and Δ*invG* strain for 48 h. SL1344 infected *cttn*$^{-/-}$ mice severely as manifested by strong weight loss, reduced intestine size, absence of faeces and reduced overall motility. Histopathological analysis of whole colon Swiss roles revealed stronger crypt erosions and increased extravasation of leukocytes in the absence of cortactin.
Therefore, we conclude that cortactin plays an important role in the restriction of *Salmonella* invasion and the maintenance of the epithelial barrier during infection. Currently, we are investigating which *Salmonella* effector protein or virulence mechanism may target cortactin and permits *Salmonella* to overcome the inhibitory functions of cortactin in this context.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

398/EEP
Microbiological assessment of *Burkholderia cepacia* complex isolates from industrial environment
K. Huth-Herms[1], A. Sisman[1], A. Spielvogel*[1], D. Oberschmidt[1], E. Uhlmann[1]
[1]*Fraunhofer - Institut für Produktionsanlagen und Konstruktionstechnik IPK, Berlin, Germany*

The genus Burkholderia is a group of heterogeneous bacterial strains isolated from different ecological niches. Their members are referred to a *Burkholderia cepacia* complex (Bcc). Originally isolated from rotten onions (BURKHOLDER 1950), particular attention has been drawn to Bcc because of its pathogenicity especially in cystic fibrosis and immunocompromised patients. Bcc is a gram negative, motile group of microorganisms and carries several intrinsic antibiotic resistances. In general, correct phenotypic and genotypic identification of Bcc is hampered by taxonomic complexity. The purpose of the presented study is to present date from Bcc bacteria isolated not from patients but from different industrial coating lines. Bcc bacteria in this environmental niche interfere with the coating process. In order to protect the coating process a high amount of biocides is usually used. During this study we isolated and identified Bcc bacteria from different process steps. Those industrial isolates were phenotypically and genotypically analysed and compared to the wild type strain (DSM7288). First results indicate that isolates display different properties, e.g. regarding growth behaviour, biocide susceptibility and biofilm formation capacity. The result of this work will help to choose the right counter-measurements and prophylaxes steps in order to prevent coating failure and to reduce biocide use.

References
[1] Burkholder, W. H. (1950): Sour skin, a bacterial rot of Onion bulbs. In: *Phytopathology* 40 (1).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

399/EEP
Title: Cable bacteria couple nitrate reduction to iron and sulfur cycles in anaerobic enrichment culture
H. Müller*[1], S. Marozava[1], T. Lueders[1], R. U. Meckenstock[2]
[1]*Helmholtz Center Munich, Institute of Groundwater Ecology, Munich, Germany*
[2]*Universität Duisburg-Essen, Aquatic microbiology, Essen, Germany*

Question: Filamentous cable bacteria of the family *Desulfobulbaceae* couple spatially-separated sulfur oxidation and oxygen or nitrate reduction by a long-distance electron transfer over centimeter distances in marine sediments [1], freshwater streams [2], and contaminated aquifers [3]. However, the function of closely related *Desulfobulbaceae* in habitats with high rates of iron reduction is presently unknown. Here, we report on the surprising detection of cable bacteria in an iron-reducing, 1-methylnaphthalene-degrading enrichment culture (1MN) and on the elucidation of their function in such habitats.
Methods: We grew culture 1MN in the presence of 1-methylnaphthalene, elemental sulfur, and FeS each with ferrihydrite and nitrate as electron acceptors in anoxic groundwater medium initially reduced with 0.3 to 2 mM sulfide. The microbial community composition was evaluated by T-RFLP, 454 pyrosequencing, and fluorescence in situ hybridization (FISH).
Results: Culture 1MN was capable of growing not only with 1-methylnaphthalene, but also with elemental sulfur and FeS as electron donor. This was accompanied with an increase of the *Desulfobulbaceae* abundance up to 95% providing strong evidence that *Desulfobulbaceae* are involved in sulfur cycling in culture 1MN. Fluorescence in situ hybridization revealed the presence of *Desulfobulbaceae* as filaments of several hundred micrometers length.
Conclusion: These cable bacteria might catalyze iron reduction in 1MN by a cryptic sulfur cycle involving sulfur disproportionation, abiotic ferrihydrite reduction, and FeS oxidation with nitrate as electron acceptor.

References
[1] Pfeffer, C., et al., Nature, 2012.
[2] Risgaard-Petersen, N., et al., AEM, 2015.
[3] Müller H, et al., The ISME journal, 2016.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Eukaryotic Pathogens (FG EK)

400/EKP
The peroxiredoxin Asp f3 protects *Aspergillus fumigatus* against external superoxide
J. M. Boysen*[1], B. Bardl[2], F. Hillmann[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Evolution of Microbial Interactions, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Bio Pilot Plant, Jena, Germany*

The ubiquitous mould *Aspergillus fumigatus* is a filamentous ascomycete with a generally saprophytic lifestyle that is also known to be an opportunistic human pathogen. Especially immunocompromised patients are at a high risk to suffer from *A. fumigatus* infections which range from allergic reactions to often fatal invasive aspergillosis (IA). Chronic granulomatous disease (CGD) results in a severe immunodeficiency based on the reduced capability to produce reactive oxygen species (ROS), and patients are at an extremely high risk to develop IA. However, it is up to now not known, how reactive oxygen species formation protect the host against fungal infections or whether these molecules target the fungus directly.
We have recently characterized the major allergen Asp f3 of *A. fumigatus*. Structural and functional studies identified Asp f3 as a two-cysteine type peroxiredoxin which showed moderate thioredoxin dependant peroxidase activity *in vitro*. Nevertheless, deletion of the *asp f3* gene led to a drastic increase in the sensitivity to ROS and the mutant was avirulent in a mouse model of pulmonary aspergillosis. We have furthermore identified six other peroxiredoxin genes whose deletion did not alter ROS sensitivity of *A. fumigatus*, suggesting that Asp f3 functions as the only major peroxiredoxin in the defence against external ROS. Since the protective mechanism of Asp f3 during host invasive growth and in the presence of ROS is unknown, we aim to elucidate its cellular function as well as the biochemical targets of ROS. To monitor their effects on *A. fumigatus*, we have established an *in vivo* assay, which allows the specific exposure to external pulses of superoxide (O_2^-), the primary product of the NADPH oxidase of innate immune cells. Surprisingly, sensitivity of the mutant was specific towards O_2^- and comparably higher than to hydrogen peroxide (H_2O_2), indicating that O_2^- causes direct damage on at least one yet-unknown cellular target. Neither O_2^- nor H_2O_2 exposure alone was lethal to *A. fumigatus*, as conidia and germlings could recover growth after longer periods of regeneration, suggesting that Asp f3 function is rather related to the repair of oxidative damage than to the direct reduction of toxic ROS. We are currently testing which metabolic pathways could serve as the primary targets of O_2^- and first results from these screening approaches will be presented.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

401/EKP
Lactobacilli prevent *Candida albicans*-mediated damage of intestinal cells in an *in vitro* commensal model
A. Dräger*[1], K. Graf[1], S. Mogavero[1], B. Hube[1]
[1]*HKI, MPM, Jena, Germany*

Question: The main reservoir for systemic *Candida albicans* infections is the gut, where the fungus exists normally as a harmless commensal interacting with the host and the microbiota. Removal or imbalance of the bacterial microbiota by antibiotic treatments can cause fungal overgrowth and is a significant predisposing factor for disseminated *Candida* infections. Our aim is to investigate the commensal-to-pathogen shift of *C. albicans via* removal of the protective bacterial microbiota and to characterize the role of non-pathogenic bacteria in keeping the fungus in the commensal stage.
Methods: We established an *in vitro* two-cell-line commensal intestinal model to study interactions between different *Lactobacillus* species and *C. albicans*. As read-outs we quantified growth dynamics, viability, adhesion and damage potential of the fungus as well as its morphology and external environmental alterations like the pH. An antibiotic treatment was used to mimic a shift from the commensal to a pathogenic state of *C. albicans*.
Results: Using the *in vitro* commensal model, we were able to show a time-, dose-, and species-dependent protective effect of lactobacilli against *C. albicans* induced cell damage. This protective effect required viable lactobacilli and the presence of host cells and is not associated with reduced fungal adherence. Further reduction of intestinal cell damage was achieved by incubating at low oxygen conditions. Moreover, it was possible to reverse the protective effect and to induce a commensal-to-pathogen shift in *C. albicans* after antibiotic treatment. Next, transcriptional profiling of *C. albicans* and intestinal cells will be used to gain detailed information about their gene expression pattern during this transition.

Conclusion: The established commensal model and our results suggest that certain *Lactobacillus* species are effective in protecting intestinal cells against damage by *C. albicans*. This requires an active interplay between viable bacteria and the host and is not due to a bacterial mediated reduction of attachment sites.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

402/EKP

Studying the role of *CgMIP1* in the evolution of *Candida glabrata* during adaption to the human host

S. Siscar-Lewin*[1], S. Brunke[1], B. Hube[1]
[1]*Hans Knoell Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*

Question: *Candida glabrata* is the second most prevalent cause of candidemia worldwide, which is largely due to its high intrinsic resistance and rapidly acquired resistance to azole antifungals. Phylogenetically, *C. glabrata* is more closely related to the bakers yeast *Saccharomyces cerevisiae* than to the most common *Candida* species, *C. albicans*. Recent evolutionary studies have shown that the gene *CgMIP1* may have been under positive selection during the evolution of *C. glabrata* as human pathogen[1]. The ortholog *MIP1* in *S. cerevisiae* encodes a mitochondrial polymerase. Defects in mitochondrial functions due to dysfunctional mitochondrial proteins are known to cause respiratory deficiency, as described in *petite* mutants, are also linked to increased resistance against azole antifungals[2]. We studied the possible role of *CgMIP1* in *C. glabrata* compared to the non-pathogenic species *S. cerevisiae.*
Methods: We created *MIP1* knock-out mutants of both species and analyzed their growth under different stress conditions, such as oxidative stress, osmotic stress, endoplasmic reticulum (ER) stress or cell wall stress.
Result: *MIP* mutants (*mip1Δ*) of both species showed reduced growth and formed small colonies under non-stress conditions, similar to the known *petite* phenotype. Interestingly, the *C. glabrata* mutant showed steady growth under ER stress conditions (tunicamycin, β-mercaptoethanol, and DTT) where growth of the wild type was notably slower. On the other hand, both *mipΔ* and wild type from *S. cerevisiae* exhibited reduced growth in these conditions. Hence, deletion of *MIP1* conferred a species-specific increased resistance to these stressors for *C. glabrata*.
Conclusion: Since the development of intrinsic drug resistance of *C. glabrata* preceded the use of antifungal drugs, these adaptations might originally be due to frequent exposure to noxious substances, e.g. originating from the mucosal flora. Such conditions might be responsible for the inferred selection pressure on *CgMIP1*, for example by allowing loss of function upon certain stressors. In *petite*-like growth, *C. glabrata* might thus better survive in these harmful environments. Studies of *C. glabrata* and *S. cerevisiae* and their mutants under different stress conditions are underway to verify this hypothesis.

References

[1] Gabaldón T, Martin T, Marcet-Houben M, Durrens P, Bolotin-Fukuhara M, Lespinet O, Arnaise S, Boisnard S, Aguileta G, Atanasova R *et al*: Comparative genomics of emerging pathogens in *Candida glabrata* clade. BMC Genomics 2013, 14:623.
[2] Tsai H-F, Krol AA, Sarti KE, and Bennett JE**:** *Candida glabrata PDR1*, a transcriptional regulator of a pleiotropic drug resistance network, mediates azole resistance in clinical isolates and *petite* mutants**.** Antimicrobial Agents and Chemotherapy 2006, 50:1384–1392

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

403/EKP

Transcription factor Ahr1 regulates expression of *ECE1* in *Candida albicans*

S. Ruben*[1], R. Martin[1], O. Kurzai[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute, Septomics Research Center, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knoell Institute, National Reference Center for Invasive Fungal Infections, Jena, Germany*

Introduction: *Candida albicans* is a commensal fungus and opportunistic pathogen. It is able to colonize and invade host tissue of immunocompromised individuals leading from superficial infections to disseminated candidiasis. The ability to switch between yeast and hyphal growth form is believed to be one of the fungus major virulence traits and is associated with the upregulated transcription of core filamentation response genes. This set of genes includes *ALS3*, *ECE1* and *HWP1*. Several transcription factors contribute to the regulation of these genes and to hyphal morphology. Recently, regulator Ahr1 was shown to be involved in several processes like white opaque switching, biofilm formation but also in fungal interaction with human immune cells. As this transcription factor is specific for *C. albicans* and close relatives, we examined if it is also contributing to activation of core filamentation response gene expression.
Objectives: As the transcription factor Ahr1 is specific for *C. albicans* and close relatives, we examined if it is contributing to hyphal morphology and activation of core filamentation response genes.
Material and Methods: We examined the morphology of mutants either lacking AHR1 or expressing a hyperactive allele where the gene was fused to a Gal4 activator domain. Consequently, qRT PCR was performed to study the expression of core filamentation response genes. We have also used a GFP reporter system where GFP was under control of the *ECE1* promoter to visualize the transcriptional dynamics of *ECE1* in dependence of Ahr1.
Results: *C. albicans ahr1*Δ mutants formed phenotypically normal hyphae in serum-containing medium. However, the transcription of *ECE1* in these mutants was significantly lower than in wild type cells. This transcription level was however higher than in wild type yeast cells. Despite this intermediate transcription of *ECE1*, *ahr1*Δ hyphae displayed no or only weak fluorescence signals when GFP was put under control of the *ECE1* promoter. In contrast, the hyperactive AHR1 allele induced high levels of *ECE1* expression even under yeast growth conditions. Additionally, it significantly induced *ECE1* expression and hyphal growth in *cph1*Δ*/efg1*Δ mutants, which are usually only able to form yeast cells even under hyphae inducing conditions.
Conclusions: The transcription factor Ahr1 seems to be required for high level transcription of the *ECE1* gene. Surprisingly, a hyperactive allele of this regulatory gene can bypass the absence of hyphal growth stimulation to induce *ECE1* transcription. This novel mechanism of *ECE1* regulation seems to be independent from the well-known regulators of hyphal growth, Cph1 and Efg1.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

404/EKP

Simultaneous acquisition of drug and antifungal peptide resistance in *Candida albicans* by gain-of-function mutations in the zinc cluster transcription factor Mrr1

I. Hampe*[1], J. Friedman[2], M. Edgerton [2], J. Morschhäuser[1]
[1]*Institute for Molecular Infection Biology, Mycology , Würzburg, Germany*
[2]*University at Buffalo, Department of Oral Biology, Buffalo, New York, United States*

Humans secrete saliva containing different antimicrobial peptides such as histatins in order to protect the oral mucosa from bacteria, fungi and other invaders. *C. albicans* can tolerate the presence of low levels of the antimicrobial peptide histatin 5 (Hst 5) because it prevents its intracellular accumulation by transporting it out of the cell *via* the Flu1 efflux pump. The expression of several other efflux pumps, which mediate antifungal drug resistance, is regulated by members of the zinc cluster transcription factor family. Gain-of-function (GOF) mutations that result in hyperactivity of these transcription factors are a frequent cause of efflux pump overexpression and drug resistance in clinical *C. albicans* isolates. We hypothesized that *FLU1* expression might also be controlled by a zinc cluster protein and that *C. albicans* could develop Hst 5 resistance by acquiring activating mutations in such a transcription factor. As cells lacking *FLU1* are also hypersusceptible to mycophenolic acid (MPA), we screened a comprehensive library of *C. albicans* strains expressing artificially activated forms of all 82 predicted zinc cluster transcription factors of this fungus for strains with increased MPA resistance. A hyperactive form of Mrr1, which is an important regulator of the multidrug efflux pump *MDR1* and responsible for fluconazole resistance in many clinical *C. albicans* isolates, upregulated *FLU1* expression and conferred MPA resistance in a *FLU1*-dependent manner. *MDR1*-overexpressing, fluconazole-resistant clinical *C. albicans* isolates containing gain-of-function mutations in *MRR1* also showed increased *FLU1* expression as well as reduced Hst 5 susceptibility, which returned to basal levels after *MRR1* deletion. Surprisingly, the Mrr1-mediated Hst 5 resistance did not depend on *FLU1*. We found that hyperactive forms of Mrr1 caused overexpression of several related efflux pumps, which all may contribute to the increased Hst 5 resistance. Our results indicate that antimycotic therapy may select for strains that have developed drug resistance and at the same time an increased ability to withstand a natural host defense mechanism.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

405/EKP

A.fumigatus infections: Strategies, metabolism and host immunity signalling

T. Dandekar*[1], S. Schuster[2], M. Dittrich[1], J. Balkenhol[1], S. Dühring[2], M. Srivastava[1], C. Remmele[1], J. Löffler[3], K. Czakai[3], M. Kaltdorf[1]
[1]*University of Würzburg, Bioinformatics, Würzburg, Germany*
[2]*University of Jena, Bioinformatics, Jena, Germany*
[3]*University Clinic of Würzburg, Internal Medicine II, Würzburg, Germany*

Question: A.fumigatus infection is only possible as A.fumigatus has clever adaptation strategies and a versatile metabolism. Another factor are specific molecular signals and interactions, which are also critical for host immune defense. We compare here both factors to understand why A.fumigatus, often a harmless saprophyte, can become a deadly pathogen in immune compromised patients.
Methods: Game theoretical modelling is used to identify different infection strategies and host counter defences. Metabolic modelling allows to translate the strategies into pathway activities and metabolic adaptations. In a second project, signalling networks are modelled and calculated using orthology relationships from different host-pathogen data and then translated into dynamical models of signalling.
Results: We show that nutrient poor conditions such as iron limitation trigger specific stress responses in A.fumigatus. However, in addition to this, further pathways help to deal with exposure to immune cells. Metabolic models for this are presented and validated using omics data sets. Furthermore, modelling of the different strategies pinpoints key elements how basic stress adaptation is turned into immune evasion and infection strategies in A.fumigatus.
The second part of the results investigates involved signalling. In particular, we look at shared A.fumigatus and human host interaction networks. Building on this, resulting immune cell signalling is modelled focussing on dendritic cell responses. For validation again different gene expression data-sets are used.
Conclusions: Our data and models show better the full range of strategies, metabolic adaptation and signalling that
A.fumigatus has at its disposal and how the host defence uses specific signalling to cope with the infection.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

406/EKP

Interaction of *Candida albicans* and *C. glabrata* with oral and vaginal epithelial cells *in vitro*

M. Pekmezovic*[1], S. Mogavero[1], B. Hube[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knoell-Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*

Question: *Candida albicans* and *C. glabrata* are the most common pathogenic *Candida* species of humans. Although they normally exist as benign commensals on mucosal sites, these *Candida* spp. can cause diseases ranging from superficial skin and mucosal infections to systemic infections when host barriers or immunity are impaired. Mixed infections of both species are not rare. While both formally belong to the genus *Candida*, they are phylogenetically very different and must have evolved their virulence potential independently. The transition from commensalism to pathogenicity occurs in three major steps: adhesion, invasion and damage of the host cells. During this transition, the host-pathogen interaction becomes a complex interplay between fungal virulence factors and the host immune response. We aimed to investigate the adhesion, invasion and damage potential of *C. albicans* and *C. glabrata,* during infection of oral and vaginal human epithelial cells (ECs) *in vitro*.
Methods: *C. albicans* and *C. glabrata* wildtype strains were used to infect human oral (TR146) and vaginal (A431 and HMV-II) ECs. Infection was done with either one (single infection) or both strains (mixed infection in a 1:1 ratio). For *C. glabrata*, we also performed infection assays with higher inoculum size as well as with sessile biofilm cells. Adhesion was measured 1 h post-infection (p.i.) and expressed as a percentage of adhered *Candida* cells to the ECs. Invasion was measured 3 h p.i., expressed as percentage of invading hyphae and hyphal length. Damage of ECs was determined 24 and 48 h p.i. by using lactate dehydrogenase cytotoxicity assay.
Results: *C. albicans* cells adhered slightly more to the ECs compared to *C. glabrata*, with the highest adherence observed on TR146 ECs. Adhesion was not increased when higher *C. glabrata* inocula were used, but we observed two times higher adhesion of biofilm cells. Invasion was not observed for *C. glabrata*, while 18-25% *C. albicans* hyphae were found to be invasive, regardless of the human cell line. Both species caused epithelial damage of all three cell types, but the damage was lower for HMV-II compared to other ECs. For mixed infections, no difference was observed compared to single infections, for any of the measured parameters.
Conclusion: Using an *in vitro* model, we compared the pathogenicity of the two most common *Candida* species during

infection of different ECs. Although lacking the ability to form hyphae, *C. glabrata* was able to cause epithelial damage without invasion, indicating the presence of a damage mechanism independent of morphological transition. For the first time, *C. glabrata* biofilm cells were tested and showed increased virulence in terms of adhesion. The specific set of adhesins expressed in biofilms will be studied in more detail. Additionally, the difference in damage between two types of vaginal ECs highlights the importance of host factors for the outcome of an infection.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

407/EKP

Incidence and Mechanism of Azole Resistance among *Aspergillus fumigatus* in Germany

A. Barber*[1], I. Schliebner[2], H. Deising[2], O. Kurzai[1]
[1]*Hans Knöll Institute, Fungal Septomics, Jena, Germany*
[2]*Martin Luther University of Halle-Wittenberg, Halle, Germany*

Introduction: *Aspergillus fumigatus* is a ubiquitous, saprophytic fungus capable of causing a life-threatening condition called invasive aspergillosis (IA) within immunocompromised populations and is particularly problematic to those receiving hematopoetic stem cell transplants, solid organ transplants, or chemotherapy. Current management strategies rely on the triazoles, a class of demethylase inhibitors that interfere with sterol biosynthesis. Alarmingly, within recent years there has been an increase in the distribution and incidence of azole resistance in *A. fumigatus* and resistant strains are now being regularly reported across the globe. Unfortunately, the mortality rate for patients infected with an azole-resistant strain is 88%.
Objectives: Azoles are heavily used for plant protection and, as such, the development of fungal resistance has been linked with agriculture. The aim of this study was to further define this relationship by surveying azole resistance among environmental *A. fumigatus* isolates in Germany and compare the resistance profiles and mechanisms present on farms practicing conventional agriculture to those utilizing organic agricultural practices.
Materials and Methods: Soil samples were collected from 11 agricultural sites in Saxony, Saxony-Anhalt, and Thuringia. *A. fumigatus* was isolated from soil using temperature selective incubation at 50°C and confirmed by morphological and molecular characteristics. Strains were examined for resistance against itraconazole (2 mg/ml), difenoconazole (2 mg/ml), prothioconazole (8 mg/ml), and tebuconazole (4 mg/ml).
Results: Analysis of 900 soil samples yielded 746 novel *A. fumigatus* isolates. Resistance testing is underway and thus far 16% (59/388) of the isolates display resistance to the medical azole itraconazole. Cross resistance to agricultural azoles was also assessed and found to be 69%, 4%, and 19% for difenoconazole, prothioconazole, and tebuconazole respectively.
Conclusions: The observed rate for itraconazole resistance among agricultural *A. fumigatus* isolates in Germany is 16%. Assessment of the molecular mechanism(s) underlying the observed resistance as well as the resistance rates of conventional compared to organic agriculture remains to be determined.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

408/EKP

The tick-borne human pathogenic parasite *Babesia* in *Ixodes ricinus* ticks - clinical and serological outcome after a tick bite in North Europe

M. Lövmar[1], P. Wilhelmsson[1], H. Vedel Nielsen[2], K. Krogfelt[2], P. Forsberg[3], P. E. Lindgren*[1]
[1]*Linköping University, Medical Microbiology, Linköping, Sweden*
[2]*Statens Seruminstitut, Copenhagen, Denmark*
[3]*Linköping University, Infectious Medicine, Linköping, Sweden*

Introduction: The *Babesia* parasites are intracellular pathogens causing human babesiosis, a tick-borne disease with malaria-like symptoms such as fever, headache, chills, myalgia and nausea. The general knowledge of *Babesia*, pathogenesis and clinical outcome in Scandinavia is very limited.
Objectives: Thus we aim to investigate the prevalence of *Babesia* spp. in ticks in Finland and Sweden, and to determine the risk of getting babesia infection, after a bite by a *Babesia*-containing tick.
Patients and methods: All sample material used in this project was previously collected in the Tick-Borne Diseases (TBD)-STING-study, in which ticks were collected at primary healthcare centers (PHCs) in Sweden and the Åland Islands, Finland, from tick-bitten persons, who also delivered blood samples and filled-in questionnaires at time of the tick bite and after twelve weeks. PCR analysis was performed on ticks collected in 2008-09. The PCR-results were confirmed by sequencing of partial *18*S rRNA genes, followed by species determination. The serum samples from tick-bitten participants bitten by *Babesia*-positive ticks were subjected to serological analysis. The questionnaires together with medical records were scrutinized for the participants bitten by *Babesia*-positive ticks as well as for serological-positive participants.
Results: 2097 *Ixodes ricinus* ticks from 1822 participants were analyzed (448 adults, 1466 nymphs, 87 larvae and 56 unidentified). 65 of the ticks were PCR-positive for *Babesia spp*, giving a prevalence of 3.0%. The species determined by sequencing, were *Babesia venatorum* (1.3%), *Babesia microti* (1.5%) and *Babesia divergens* (0.2%). Furthermore, 30/65 ticks found positive for *Babesia spp*, were positive for Borrelia spp, in a real-time PCR assay, thus giving a co-infection rate of 40%. Seven participants were serologically positive, with antibody titers ranging from 1:64 to 1:256. There were five individuals reporting symptoms in their questionnaires, one of which was also serologically positive. However it is not clear that these symtoms are related to babesiosis.
Conclusion: Taken together, this is the first general survey in Nordic Countries of the prevalence of *Babesia spp* in ticks detached from humans related to serological and clinical outcome of the tick-bitten person. Thus, the risk of contracting an infection after a tick bite of a *Babesia*-containing tick seems to be very low. However, since we notice some participants with a previous serological response to *Babesia*, babesiosis should not be overlooked as a potential diagnosis in patients showing flu-like symptoms after a tick bite
The authors want to acknowledge the EU-Interreg project ScandTick Innovation for financial support.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

409/EKP

Hypoxia influences the *Candida albicans* – enterocyte interaction

N. Engert*[1], R. Mikolajczyk[1], E. Gressler[1], I. D. Jacobsen[1,2,3]
[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute Jena, Research Group Microbial Immunology, Jena, Germany*
[2]*Friedrich-Schiller University, Jena, Germany*
[3]*Center for Sepsis Control and Care (CSCC), University Hospital Jena, Jena, Germany*

The opportunistic fungal pathogen *Candida albicans* frequently occurs as commensal in the gastrointestinal tract of humans. While it is known that intestinal epithelial cells are highly sensitive to ischemic hypoxia, the role of hypoxia-mediated damage in the translocation of *C. albicans* through the intestinal barrier, potentially leading to life-threatening systemic infections, is unclear. Thus, we characterised enterocyte-*C. albicans* interactions under normoxic and hypoxic conditions.

When enterocytes were shifted from low to higher O_2 levels (reperfusion) for the time of infection, *C. albicans*-mediated damage increased significantly, especially for O_2 levels ≤ 2 %. Surprisingly, shifting the cells from ambient or physiological conditions to lower O_2 levels (hypoxic shock) immediately before infection led to reduced damage. Subsequent reperfusion after exposure to hypoxic shock (1 % O_2) for 2 h abolished the protective effect of hypoxic shock and increased damage of infected enterocytes. Supporting these data, the invasion potential of *C. albicans* and the permeability of infected enterocytes was exclusively increased at reperfusion whereas hypoxic shock conditions increased fungal adhesion. Consistent with these results, trans-epithelial electrical resistance (TEER) remained stable during infection at hypoxic shock but decreased continuously for all other conditions. TEER values for uninfected enterocytes showed no significant differences between the tested O_2 conditions. Strikingly, ROS levels of both infected and non-infected enterocytes were increased at hypoxic shock conditions but not at reperfusion.

In ongoing experiments we aim to analyse the infection process in more detail to determine the mechanisms that lead to increased damage during reperfusion. This includes analysis of the expression of tight junction proteins, the role of hypoxia-inducible factor 1 (HIF-1α) for protection during hypoxic shock and transcriptional profiling to identify further O_2-induced changes in enterocytes contributing to infection susceptibility.

We have furthermore begun to analyse the adaptation of *C. albicans* to different O_2 levels during interaction with host cells. Here, we observed minor but significant reduction in hyphal length, which could contribute to but does not fully explain the reduced damage observed during hypoxic shock. Deletion of most fungal factors known to govern the fungal response to hypoxia, such as *EFG1*, *ACE2* and *UPC2*, led to reduced epithelial damage independent of the O_2 level and was associated with reduced growth rates in cell culture media. In contrast, a *tye7*ΔΔ mutant displayed O_2-dependent virulence with significantly enhanced enterocyte damage at ≥ O2 but significant attenuation at 1% O_2 and hypoxic shock. This suggests that both host susceptibility and fungal adaptation are influenced by the O_2 level during infection.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

410/EKP

The dual function of *Candida albicans* Ece1 during interaction with macrophages: damage, escape and activation of pro-inflammatory pathways.

A. Franke*[1], L. Kasper[1], S. Mogavero[1], B. Hube[1,2,3]
[1]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI) , Microbial Pathogenicity Mechanisms, Jena, Germany*
[2]*Center for Sepsis Control and Care, University Hospital, Jena, Germany*
[3]*Friedrich Schiller University, Jena, Germany*

Question: *Candida albicans*, an opportunistic human pathogen, uses different strategies to evade or adapt to phagocyte antimicrobial activities and to survive immune cell uptake. Phagocytosis of *C. albicans* yeast cells by cultured macrophages induces intracellular filamentation, which results in escape of the fungus from these immune cells and host cell damage. In addition, hypha formation is connected with induction of pro-inflammatory cytokines in these immune cells.

We investigate *ECE1*, a hypha-associated gene, which is highly up-regulated during filamentation of *C. albicans*. The encoded protein Ece1 is a polypeptide comprised of eight peptides (I-VIII), of which peptide III (Candidalysin) is capable of producing lesions in host cell membranes [1]. Besides Candidalysin, almost all other Ece1 peptides are secreted upon hypha formation [1]. We are focusing on the role of non-Candidalysin Ece1 peptides during the interaction of *C. albicans* with host cells.

Methods: The secretion of Ece1 peptides is determined by LC/MS analysis in the supernatant of *C. albicans* cultures grown under hypha-inducing conditions. Cytokine induction by macrophages upon *C. albicans* infection or co-incubation with synthetic peptides is monitored by ELISA and Western Blot. As a read-out for infectivity of single peptide knockout mutants, damage of macrophages is analyzed by measuring the release of cytoplasmic lactate dehydrogenase upon fungal infection.

Results: The Ece1 protein itself is dispensable for phagocytosis of *C. albicans*, subsequent filamentation in and hyphal outgrowth from macrophages. However, the secreted peptide toxin Candidalysin is involved in immune cell death and NLRP3-inflammasome activation.

Preliminary data show that peptide V, one of the most abundant secreted Ece1 peptides, induces pro-inflammatory cytokine induction by macrophages, thus highlighting the importance of non-Candidalysin peptides during the fungus-host interaction.

Conclusion: We propose a dual function of Ece1: Candidalysin-mediated lesion formation is beneficial for *C. albicans* immune evasion by providing an escape route for the fungus, but might as well contribute to antifungal host defense through activation of pro-inflammatory pathways.

Non-Candidalysin Ece1 peptides might facilitate the Candidalysin action, but might as well possess an independent role during the infection process.

Reference

[1] Moyes *et al.* 2016, Candidalysin is a fungal peptide toxin critical for mucosal infection, *Nature* 532, pp. 64–68

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

411/EKP

The Snf1-activating kinase Sak1 is essential for metabolic adaptation and *in vivo* fitness of *Candida albicans*

B. Ramirez-Zavala*[1], A. Mottola[1], S. Schneider[1], J. Haubenreißer[1], S. Allert[2], S. Brunke[2], B. Hube[2], K. Ohlsen[1], J. Morschhäuser[1]
[1]*University of Wuerzburg, Institute for Molecular Infection Biology, Wuerzburg, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Department of Microbial Pathogenicity Mechanisms, Jena, Germany*

The yeast *Candida albicans* is a harmless commensal of mucosal surfaces in healthy people, but can also cause superficial as well as life-threatening systemic infections in immunocompromised hosts. The ability of *C. albicans* to adapt to changing environmental conditions within the host is important for the survival and proliferation of the fungus. *C. albicans* has developed metabolic plasticity and can utilize many alternative carbon sources, such as lactate that is present in various host niches. The Snf1/AMPK signalling pathway is highly conserved in eukaryotes and plays a fundamental role in the response to nutrient stress. Although this pathway has been extensively studied in the yeast *Saccharomyces cerevisiae*, little is known about its function in *C. albicans*. One of the reasons is that, contrary to *S. cerevisiae*, several components of the Snf1 complex are essential in *C. albicans*. As part of a comprehensive study of the *C. albicans* kinome, we identified a putative kinase (orf19.3840) whose deletion caused increased sensitivity towards cell wall stress as well as filamentation defects and impaired growth on alternative carbon sources. The ortholog of orf19.3840 in *S. cerevisiae* (Sc*SAK1*) encodes one of three partially redundant upstream kinases that activate Snf1 by phosphorylation at Thr210. Hence, we conducted a functional analysis of the Snf1 complex in *C. albicans*. Our results showed that Snf1 phosphorylation at the conserved residue Thr208 depends on orf19.3840, encoding CaSak1, and that this modification is essential for its proper activation. Hyperactive mutant forms of Snf1 partially rescued the *sak1*Δ defects. *C. albicans* mutants lacking the regulatory subunit Snf4 showed more severe phenotypes than *sak1*Δ mutants, although Thr208 in Snf1 was still phosphorylated. Transcriptome analysis of *sak1*Δ mutants showed decreased expression of several glucose transporters, explaining the slightly reduced growth even in the presence of the preferred carbon source glucose. Conversely, genes related to cell wall biogenesis were upregulated, reflecting the cell wall defects of the *sak1*Δ mutants. When tested in a mouse gastrointestinal colonization model, the *sak1*Δ mutants were rapidly outcompeted by the wild type parental strain. These results demonstrate that Sak1 has a key role in the activation of the Snf1 pathway in *C. albicans* and is essential for the metabolic adaptation and *in vivo* fitness of the fungus.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Fungal Biology (FB)

412/FBP

Investigation of protein interactions to elucidate the function of unknown proteins in the human pathogenic fungus *Aspergillus fumigatus*

M. Nossmann*[1], F. Hillmann[2], A. Brakhage[3,4], T. Munder[1]
[1]*Ernst-Abbe-University of Applied Sciences Jena, Medical Engineering and Biotechnology, Jena, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Evolution of Microbial Interactions, Jena, Germany*
[3]*Friedrich Schiller University Jena, Institute of Microbiology, Jena, Germany*
[4]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Molecular and Applied Microbiology, Jena, Germany*

The saprophytic fungus *Aspergillus fumigatus* is considered as the most prevalent and dangerous airborne fungal pathogen. Because of its ability to survive in a broad temperature range from 12°C to 56°C the fungus is distributed all over the world causing several respiratory allergies, contributing to e.g. asthma and allergic sinusitis. Its conidia are able to reach the alveoli and cause invasive infections in immunocompromised patients, with mortalities up to 90 %.
Genome and transcriptome data predict roughly 10.000 genes, the vast majority without a known function. Especially in regard to its multifactorial virulence, the elucidation of protein function and the investigation of molecular pathways are of great importance. The yeast two hybrid technology is powerful tool to identify novel protein/protein interactions. The modular structure of transcription factors is used to identify specific protein binding partners through a reporter system to detect their interaction. Within the two hybrid system, the transcription factor is a dimeric protein consisting of a DNA binding domain (DBD) and an activation domain (AD). When bait and prey proteins interact, the DBD and AD are brought into proximity to activate the transcription of independent reporter genes. This technique was limited by the inefficient expression of *A. fumigatus* proteins in the baker"s yeast *Saccharomyces cerevisiae.* We hypothesized that the low protein yield resulted primarily from differences in the codon usage of *S. cerevisiae* and *A. fumigatus* which would result in a lack of codon specific tRNA molecules in yeast, and hence lead to low protein yield. To solve this problem, we generated a tRNA adapted strain of *S. cerevisiae* which allowed a highly efficient translation of *A. fumigatus* mRNAs. As a proof-of-principle we exploited this codon-adapted yeast strain to screen for protein interactions with a highly expressed, but functionally unknown gene product (SmhA) of *A. fumigatus* against an expression library based on *A. fumigatus* cDNA.
We found a putative pyruvate decarboxylase (PdcA) as a potential interaction partner with SmhA. Interestingly, both interaction partners are highly expressed during hypoxia. A gene deletion mutant of *smhA* confirmed a functional role of this protein during growth in low oxygen atmospheres. We are currently performing further *in vitro* analyses as well as interaction studies in *A. fumigatus* to verify a functional interaction with PdcA as well as other potential interaction partners. Finally, we aim to extend this system to unravel protein interaction networks during invasive growth and antifungal therapy.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

413/FBP

Pathogenic yeasts express virulence determinants to defend against predation by the mycophagous amoeba *Protostelium fungivorum*

S. Novohradska*[1], R. Tóth[2], S. Brunke[3], A. Gáscer[2], G. Glöckner[4,5], J. Linde[6], F. Hillmann[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Evolution of Microbial Interactions, Jena, Germany*
[2]*University of Szeged, Department of Microbiology, Szeged, Hungary*
[3]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Microbial Pathogenicity Mechanisms, Jena, Germany*
[4]*University of Cologne, Institute for Biochemistry, Köln, Germany*
[5]*Leibniz Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany*
[6]*Leibniz Institute for Natural Product Research and Infection Biology - Hans Knöll Institute, Systems Biology and Bioinformatics, Jena, Germany*

The prevalence of systemic mycoses caused by pathogenic yeasts is dramatically increasing. The leading causative agents are yeasts from the genera *Candida* and *Cryptococcus.* Their virulence determinants originated according to the specific niche of each organism. While some fungal pathogens like *C. albicans* are already adapted to commensal lifestyle, other yeasts such as *Cryptococcus, C. tropicalis* or even *C. parapsilosis* have also been isolated from non-human sources and their virulence might have been shaped in part outside the human host as a strategy to counteract environmental predators. The selection pressure these impose could have led to the generation of survival strategies that contributed to the virulence potential when later confronted with immune cells.
To test this hypothesis, we have isolated and sequenced a novel species of professional mycophagous amoeba, *Protostelium fungivorum*, a genus widely spread in nature, feeding exclusively on fungi. To exploit this amoeba as a new model system, we have first screened a broad prey spectrum within the fungal kingdom, including yeasts from the basidiomycetes and ascomycetes, as well as some filamentous fungi. We found that nearly all of them could serve as food sources, indicating, that most environmental fungi face predation pressure by mycophagous amoeba. *Rhodotorula mucilaginosa* and *C. parapsilosis* served as the preferred food sources, being ingested, processed via phagolysosomes, and killed within less than 10 min. Although the efficiency of amoeba killing exceeded 99%, a slight fraction of cells showed the ability to survive the predation via reversible transition into hyphae, which allowed the escape from killing and secured the survival of a stable subpopulation.
As killing and digestion were most effective with *C. parapsilosis*, we used this pathogen in a dual-transcriptome approach to identify the targets of predatory-prey interactions. Interestingly, amoeba responsive genes included those involved in the intracellular mobilization of metal resources, response to oxidative stress, filamentous growth and a hydrolytic enzyme important for pathogenicity. We have recently constructed deletion mutants for the most promising targets and their phenotypes will reveal if traits that have originated to counteract with phagocytic predators in natural environments could also have supported resistance against innate immune cells.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

414/FBP

Analysis of the role of opsins in *Alternaria alternata*

C. Pinecker*[1], U. Terpitz[2], R. Fischer[1]
[1]*Karlsruher Institut für Technologie, Institut für angewandte Biowissenschaften: Mikrobiologie, Karlsruhe, Germany*
[2]*Universität Würzburg, Lehrstuhl für Biotechnologie und Biophysik, Würzburg, Germany*

Rhodopsins are a group of proteins that occur in all domains of life. They consist of the protein (Opsin) and an all-trans retinal chromophore which binds via a protonated Schiff-base to a crucial lysine. Microbial rhodopsins of type I, occur in archaea, bacteria and lower eukaryotes like fungi. Whereas in archaea the function of halorhodopsin for energy conservation is well-studied, the function of fungal rhodopsins still remains unknown. Many fungi contain putative opsin proteins, although in many the crucial lysine residue appears not to be conserved and thus chromophore attachment is unlikely.
The filamentous fungus *Alternaria alternata* is an ascomycete that colonizes many different organic substrates and is also able to infect different plant species such as cereals and thus causes tremendous economic damage every year. We identified two rhodopsin homologues in *A. alternata*, OpsA and OpsB, which both belong to the proton pumping subgroup called bacteriorhodopsins. Their aminoacid sequences show the conserved seven transmembrane helices, the crucial lysine for retinal binding in helix G (Ernst et al. 2014) as well as the two conserved aspartic acids in helix C that are necessary for proton transport. OpsA and OpsB were expressed in mammalian cells and analyzed using a patch clamp technique. Both proteins showed proton pump activity when illuminated with green light. This resembles the findings *Fusarium fujikuroi* (García-Martínez et al. 2015). To assign a function of these rhodopsins in *A. alternata*, we established the CRISPR Cas technology and inactivated *opsA*. The *opsA*-knockout strain showed an increase in spore production and an altered secondary metabolite profile. Inactivation of *opsB* is under way. These results suggest a role of green light in *A. alternata*. This is rather exceptional, given that most fungi sense either blue- or red light (Yu et al, 2016).

References

[1] Ernst, O. P., Lodowski, D. T., Elstner, M., Hegemann, P., Brown, L. S., & Kandori, H. (2014). Microbial and animal R´rhodopsins: structures, functions, and molecular mechanisms. Chem. Rev., 114(1), 126–163.
[2] García-Martínez J., Brunk M., Avalos J., Terpitz U. (2015). The CarO rhodopsin of the fungus Fusarium fujikuroi is a light-driven proton pump that retards spore germination. Sci. Rep.5:7798.
[3] Yu Z., Armant O., Fischer R. (2016). Fungi use the SakA (HogA) pathway for phytochrome-dependent light signalling. Nat. Microbiol. 1:16019

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

415/FBP

Low-Temperature Plasma Interaction With Indoor Fungi

C. Gehrmann-Janssen*[1], K. Petersen[1]
[1]*HAWK Hildesheim, Faculty of Preservation of Cultural Heritage, Hildesheim, Germany*

Fungi occurring in indoor environments on building materials as well as on paper and textile with respect to specimen of great significance in conservation and restoration are still subjects to investigations on low-temperature plasma treatments. This study concentrates on various indoor fungi such as Aspergillus niger, A. restrictus, A. versicolor, Eurotium chevalieri, E. herbariorum, Penicillium aurantiogriseum, P. brevicompactum, P. chrysogenum, P. citrinum, Chaetomium globosum, Cladosporium

herbarum, C. sphaerospermum, Engydontium album, Stachybotrys chartarum and Ulocladium sp. to investigate their resistance against plasma operations.The effects of the plasma treatments on the viability of the fungi tested with hyaline conidia is visualized by fluorescence microscopy using the fluorochromes calcofluor white and propidium iodide. Propidium iodide penetrates only cells with damaged membranes while calcofluor white is a non–specific fluorochrome that binds to cellulose and chitin cell walls. The application of calcofluor white in combination with propidium iodide present a rapid method to enumerate live/dead cells. These assays indicate the efficacy of plasma treatments in killing the fungal cells. Fungal specimen with brown to black conidia undergo a growth (cultivation) study after plasma treatment. The germinating conidia are enumerated by light microscopy. Plasma power and treatment time used are discussed in relation to viable and non-viable fungal cells. In this context the centre of interest is the treatment time when the cells are dead nearly 100 % by the plasma. Therefore the approach is focused on a few time intervals from the bottom up and top down. A continuous dependence of the cell damage from the treatment time respectively the plasma power is momentary not tested in this study.

References

[1] Helmke, A., Grünig, P., Fritz U-M., Wandke D., Emmert, S., Petersen, K. and Vioel W. Low-temperature Plasma - a Prospective Microbicidal Tool Recent Patents on Anti-Infective Drug Discovery, 2012, 7, 223-230

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

416/FBP

Identification of the biological role of the RNA-binding protein Khd4 during pathogenic development of the plant pathogen *Ustilago maydis*

J. Lee*[1], C. Haag[1], E. Vollmeister[1,2], M. Feldbrügge[1]
[1]*Heinrich-Heine-University Düsseldorf, Institute of Microbiology, Düsseldorf, Germany*
[2]*Phillips-University Marburg, Institute for Lung Research, Marburg, Germany*

The phytopathogenic fungus *Ustilago maydis* is a useful model organism to study host-pathogen interactions with its host *Zea mays*, in which it causes corn smut disease. As a prerequisite for pathogenicity, *U. maydis* has to undergo mating, which results in a switch from yeast-like to hyphal growth. We are investigating the role of RNA-binding proteins (RBPs) during pathogenic development of *U. maydis*. Deletion of the gene encoding the RBP Khd4 results in aberrant cell morphology, reduced growth rate and severely reduced virulence. The protein belongs to a group of fungal multi hnRNP K-homology domain (KH) proteins. Khd4 contains five KH domains and recognizes the hexanucleotide AUACCC as binding motif. Initial experiments suggest a potential role for the protein in the regulation of mRNA stability. Nevertheless, the precise role of Khd4 has not yet been identified. In order to understand the biological role of Khd4, we are aiming to identify target mRNAs of this RBP by indirect means. Direct identification of bound mRNAs was shown to be problematic by preliminary work, therefore RNA-Seq and ribosome-profiling will be conducted to gain a transcriptomic-wide view of altered mRNA abundance and translation. Potential targets should be identifiable by changes in abundance and translation as well as the presence of the binding motif. These approaches will provide new insights into post-transcriptional regulation in fungi.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

417/FBP

Proteomic studies on the degradation of DHN melanin, a fungal virulence factor

A. Pschibul*[1,2], T. Krüger[1,2], O. Kniemeyer[1,2], A. A. Brakhage[1,2]
[1]*Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e. V. – Hans-Knöll-Institut, Molekulare und Angewandte Mikrobiologie, Jena, Germany*
[2]*Friedrich Schiller University, Institute of Microbiology, Jena, Germany*

Fungal melanins have a wide range of roles in fungal pathogenicity. For example, the layer of dihydroxynaphthalene (DHN) melanin located on the outside of spores of the mould *Aspergillus fumigatus* has been reported to be involved in masking immunogenic structures on the spore surface[1]. It has also been shown to reduce phagolysosomal acidification[2] and to inhibit apoptosis of macrophages after phagocytosis of the spores[3]. In addition, it contributes to the quenching of reactive oxygen species produced by host immune cells as a defence mechanism[4]. The biosynthesis of DHN-melanin has been studied in detail and enzymes involved in each step of the process have been elucidated. The only exception is the final step of biosynthesis, the polymerisation of 1,8-DHN to DHN melanin. By contrast, nothing is known about the degradation of DHN melanin, which is important for understanding of germination of conidia *e.g.* in phagolysosomes or to study the immunological impact of breakdown products. In this study, a proteomics approach was employed in order to identify enzymes with a possible role in DHN-melanin degradation. For this purpose, the proteome of *A. fumigatus* grown in the presence of 1,8-DHN was analysed. Both 2D gel electrophoresis and gel-free liquid chromatography-mass spectrometry (LC-MS/MS) were employed. A comparison of the proteins formed in the presence of 1,8-DHN and a control showed a specific response to the stress created by the presence of 1,8-DHN. Significantly enriched categories of differentially expressed proteins according to GO ontology were, for example, oxidoreductase, nucleotide binding, transferase and ATP binding activity. Some of these enzymes may be involved in DHN-melanin degradation. These are for example oxygenases, which could assist in initial steps of the degradation process. Further studies are planned in order to elucidate the role of these enzymes and to obtain more information on the degradation process of DHN-melanin.

References

[1] Bayry, J., et al., *Surface structure characterization of* Aspergillus fumigatus *conidia mutated in the melanin synthesis pathway and their human cellular immune response.* Infect Immun, 2014. 82(8):3141-53.
[2] Thywissen, A., et al., *Conidial Dihydroxynaphthalene Melanin of the Human Pathogenic Fungus* Aspergillus fumigatus *Interferes with the Host Endocytosis Pathway.* Front Microbiol, 2011. 2: 96.
[3] Volling, K., et al., *Phagocytosis of melanized* Aspergillus *conidia by macrophages exerts cytoprotective effects by sustained PI3K/Akt signalling*. Cell Microbiol, 2011. 13(8):1130-48.
[4] Wheeler M., Bell A., *Melanins and their importance in pathogenic fungi.* Curr Top Med Mycol, 1988. 2:338-87.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

418/FBP

The STRIPAK complex is essential for septation in the fungus *Sordaria macrospora*

D. Radchenko*[1], U. Kück[1]
[1]*Ruhr-University Bochum, Allgemeine und Molekulare Botanik, Bochum, Germany*

The striatin-interacting phosphatases and kinases (STRIPAK) complex is a highly conserved protein complex that was described for diverse lower and higher eukaryotes with the exception of plants. STRIPAK and STRIPAK-like complexes regulate numerous cell functions, such as proliferation and cytokinesis. In humans, STRIPAK was shown to correlate with medical conditions such as cavernous cerebral malformation, diabetes and heart diseases. In filamentous fungi, subunits of this complex control sexual development, vegetative growth, and hyphal fusion [1]. Here, we investigate the structure and function of the STRIPAK complex from a model fungus, the ascomycete *Sordaria macrospora*.

S. macrospora STRIPAK consists of protein phosphatase 2A (PP2A) scaffolding and catalytic subunits, striatin homolog PRO11, striatin-interacting protein PRO22, kinase activator MOB3, and transmembrane protein PRO45. Recently, homologs of germinal center kinases of the STE20 kinase family (GCKIII kinases) KIN3 and KIN24 were also described to be associated with STRIPAK [2, 3]. In this study, the link between KIN3 and KIN24 from *S. macrospora* with STRIPAK but also with the septation initiation network (SIN) is investigated.

SIN is another conserved protein complex, which is known in human and *Drosophila* as HIPPO. These evolutionary conserved complexes control septum formation in fission yeast and filamentous fungi, and cell proliferation in animals [4, 5, 6]. BLAST analysis provides evidence for the presence of all known SIN homologs in *S. macrospora*. The hypothetical model of the STRIPAK/SIN interaction in this fungus is presented for the first time. To support the bioinformatic analysis, double deletion strains of Hippo homolog KIN3 and striatin homolog PRO11 were generated. Double mutants Δkin3/pro11 and Δkin3/Δpro11 show an arrest at early stages of sexual development together with severe septation defects. Phenotypic differences, compared to other available STRIPAK double mutants, led us conclude that both proteins are part of different pathways. Microscopic investigation of pro11 and Δpro11 single mutant strains show the significance of the core STRIPAK protein PRO11 for septation in ascogonial and vegetative hyphae. This data further support our working hypotheses that STRIPAK is connected with the SIN complex.

References

[1] Hwang and Pallas 2014 Int J Biochen Cell B 47:118-148
[2] Kück et al. 2016 Fungal Genet Biol 90:31-38
[3] Frey et al. 2015 PloS one 10(9):e0139163
[4] Pan 2007 Gene Dev 21(8):886-897
[5] Krapp and Simanis 2008 Biochem Soc Trans 36(3):411-415
[6] Heilig et al. 2013 PloS one 8(10):e79464

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

419/FBP

Xylose-induceed production of Enzymes with modified *Aspergillus nidulans* strains

P. Ballmann*[1], M. Müller[1], S. Dröge[1], R. Prade[2]
[1]*Test- and Research Institue Pirmasens, Biotechnology & Microbiology, Pirmasens, Germany*
[2]*Oklahoma State University, Microbiology and Molecular Genetics, Stillwater, Oklahoma, United States*

Renewable raw materials play an important role in the context of the climate change. Today, biomasses of the first generation such as corn or maize dominate the market, but the demand of lignocellulose as an less controversial feedstock will growth. The using of lignocellulose assumes two major processing steps. First, a pretreatment of the biomass in a thermochemical process with diluted acid. In the second step, the solid fraction treated in an enzymatic hydrolysis to release the glucose. A couple of different cellulolytic enzymes such as Endoglucanase, Exoglucanase, and β-Glucosidase are needed. During the first pretreatment step, most of the hemicellulose dissolved in the liquid phase as xylose. The usage of xylose as the substrate is problematic because most of the relevant industrial biotechnology processes could not utilize effectively the xylose. Furthermore, a couple of inhibitor substance also released in the liquid phase. Both limit the possible applications of the liquid phase. Our goal was to use the liquid phase to produce the cellulolytic enzymes for the enzymatic hydrolysis.

Diverse species of fungal are commonly used to produce cellulolytic enzymes in the industrial scale, such as *Trichoderma reseei*, *Aspergillus niger* or *Aspergillus nidulans*. The production and release of cellulolytic enzymes typically induced by cellulose or lactose. Monosaccharides like Glucose or Xylose could not induce the expression of the enzymes. Consequently, strains of *Aspergillus nidulans* were genetical modified in this project to use xylose as an inducer for cellulolytic enzymes production. To increased generally the enzymes activity the regulation of the gene expression was also modified. Three different strains with single Endoglucanase-, Exoglucanase- and ß-Glucosidase-Activity were constructed. Components of a defined mineral medium were added to the medium. The modified strains were cultivated in a solid-state fermentation with the pretreated wheat straw as growth matrix.

The results showed that the modified *Aspergillus nidulans* strains grow very fast in the liquid hydrolysate. After three days, all sugars were consumed. The fungal formed a close mycell and high cellulolytic enzymatic activities were measured. Compared to the wild-type strain the measured enzymatic activities by all constructed strains were higher, such as a ten-time higher Endoglucanase-Activity, forty-six times higher β-Glucosidase-Activity and a double up Exoglucanase-Activity. All three strains also had a huge increased Xylanase-Activity. No negative effects of inhibiting substance in the liquid hydrolysate were observed.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

420/FBP

Virulence attributes in the interaction between *Lichtheimia corymbifera* and alveolar macrophages

M. Abdelwahab Hassan*[1], V. Schwartze[1], H. R. Park[1], B. Hanf[2], G. Nagy[3], î Csernetics[3], T. Papp[3], T. Krüger[2], Z. Cseresnyés[4], M. Thilo Figge[4], O. Kniemeyer[2], A. A. Brakhage[2], K. Voigt[1]
[1]*Leibniz-Institute for Natural Product Research and Infection Biology, Jena Microbial Research Collection, Jena, Germany*
[2]*Leibniz-Institute for Natural Product Research and Infection Biology, Molecular and Applied Microbiology, Jena, Germany*
[3]*Faculty of Science and Informatics, University of Szeged, , Department of Microbiology, Szeged, Hungary*
[4]*Leibniz-Institute for Natural Product Research and Infection Biology, Applied systems biology, Jena, Germany*

Mucoralean fungi can cause mucormycosis, a life-threatening disease in immunocompromised patients. In our study, we analysed the influence of different enzymatic treatments of the spore surface alterations on the phagocytosis by murine alveolar macrophages. Two strains which were shown to be virulent and attenuated in avian, invertebrate and murine infection models were used in this study. The spore surface was treated with different cell wall-degrading cell wall enzymes targeting carbohydrate and protein cell wall components. The highest phagocytosis index was achieved with the proteolytic treatments which encouraged us to do focus our research on the protein surface of spores. Proteomic analysis of the spore surface was conducted for both strains. About four-teen candidate proteins were found which were differentially abundant in either the virulent strain or in the attenuated strain leading to the hypothesis that these proteins may play a role in virulence. One of these candidate proteins is the hydrophobic surface binding protein A (HsbA) which was first found in higher abundance in the virulent strain of *L. corymbifera*. HsbA was first described as an adhesin in *Aspergillus oryzae*. Additionally, HsbA was purified from the insect-killing ascomycete *Beauveria bassiana* and identified to play a role in immunogenicity provoking an immune response in the host insect. The HsbA protein from the virulent strain of *L. corymbifera* was heterologously overexpressed in *Pichia pastoris*. After pretreatment of murine alveolar macrophages and spores with purified fractions of the HsbA protein, the phagocytic index was found to be enhanced in comparison with unstimulated host cells. The findings presented in this study will open the door for the role of surface protein in the recognition of *L. corymbifera* by phagocytes of the innate immune system which raise important measures to mammalian infection models. Our prospect for the future research will focus on the identification of potential stimulatory effects of *L. corymbifera* surface proteins and their putative receptors on the surface of macrophages which possibly contribute to virulence.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

421/FBP

Gene network reconstruction for *Aspergillus niger* based on transcriptomic data for 155 different cultivation conditions

P. Schäpe*[1], S. Lenz[1], B. M. Nitsche[1], V. Meyer[1]
[1]*TU Berlin, Applied and Molecular Microbiology, Berlin, Germany*

The genome of the industrial cell factory *Aspergillus niger* was published in 2008; however, up until now only 2% the genes have been experimentally investigated in the lab. For 50% of the predicted ORFs valid functional predictions based on sequence have been made, leaving about 50% of the genome with questionable predicted functions or even no prediction at all. Wet lab experiments and manual curation are time consuming and not feasible to deploy on the whole genome. To overcome this problem we suggest complementing the existing methods for genome annotation with another fast and cheap in-silico method, which is not based on sequences, but on expression data. The underlying hypothesis of this approach is that genes within shared expression profiles are predicted to function in the same regulatory and/or functional pathway (the so called "guilt-by-association" approach).
Consequently we have established a transcriptomics database using Affymetrix microarray data published for *Aspergillus niger*. The database includes 155 different cultivation conditions reflecting different carbon and nitrogen sources, starvation and stress conditions, conditions related to temporal and spatial stages during its life cycle, different cultivation concepts and many more. In order to find genes with a consistent, correlated expression pattern across phenotypically diverse samples or experimental conditions, we deployed statistical correlation measures on the global database. The resulting co-expression network was broken down into subnetworks, depicting functional modules. These functional modules were analyzed for content consistency and validated based on published data for the function of known secretory pathway genes.
These quality control analyses supported the biological relevance of the modules and subnetworks, suggesting that the co-expression network obtained presents a valuable predictive tool for functional annotation of *Aspergillus niger* genes. Most importantly, this prediction tool can also be used to improve the functional annotation of orthologous genes in other Aspergilli relevant as cell factories or known as pathogens.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

422/FBP

The sole introduction of two single point mutations establishes glycerol utilization in *Saccharomyces cerevisiae* CEN.PK derivatives

P. W. Ho*[1], S. Swinnen[1], J. Duitama[2], E. Nevoigt[1]
[1]*Jacobs University Bremen, Bremen, Germany*
[2]*Universidad de los Andes, Systems and Computing Engineering Departament, Bogotá, Colombia*

Glycerol is an abundant by-product of biodiesel production and has several advantages as a substrate in biotechnological applications. Unfortunately, the popular production host *Saccharomyces cerevisiae* can barely metabolize glycerol by nature. In this study, two evolved derivatives of the strain CEN.PK113-1A were created that were able to grow in synthetic glycerol medium (strains PW-1 and PW-2). Their growth performances on glycerol were compared with that of the previously published evolved CEN.PK113-7D derivative JL1. As JL1 showed a higher maximum specific growth rate on glycerol (0.164 versus 0.127 h-1), its genomic DNA was subjected to whole genome re-sequencing. Two point mutations in the coding sequences of the genes *UBR2* and *GUT1* were identified to be crucial for growth in synthetic glycerol medium and subsequently verified by reverse engineering of the wild-type strain CEN.PK113-7D. The growth rate of the resulting reverse-engineered strain was 0.130 h-1. Sanger sequencing of the *GUT1* and *UBR2* alleles of the above-mentioned evolved strains PW-1 and PW-2 also revealed one single point mutation in these two candidate genes, and both mutations were demonstrated to be also crucial and sufficient for obtaining a maximum specific growth rate of ~0.120 h-1 on glycerol. The current work confirmed the importance of *UBR2* and *GUT1* as targets for establishing glycerol utilization in strains of the CEN.PK family. In addition, it is shown that a growth rate on glycerol of 0.130 h-1 can be established in reverse-engineered CEN.PK strain by solely replacing a single amino acid in the coding sequences of both Ubr2 and Gut1.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer

423/FTP

Radiosensitizing effects upon *Streptococcus pyogenes* arginine deiminase based therapy of glioblastoma multiforme

C. Maletzki[1], Y. Rosche[2,1], A. Scholz[2,1], M. Linnebacher[1], B. Kreikemeyer[2], T. Fiedler*[2]
[1]*Rostock University Medical Centre, Molecular Oncology and Immunotherapy Group, Rostock, Germany*
[2]*Rostock University Medical Centre, Institute for Mecial Microbiology, Virology and Hygiene, Rostock, Germany*

Background: Arginine auxotrophy constitutes a weak point of several tumors, among them glioblastoma multiforme (GBM). In a previous study, patient-individual GBM models were found to be sensitive towards the arginine-depleting enzyme arginine deiminase (ADI) of *Streptococcus pyogenes*. Here, we aimed at improving the therapy regimen by combining ADI with several antineoplastic drugs and evaluating effects on radiosensititvity.
Methods: ADI-sensitive GBM cell lines (n=5, including a matched pair of primary [HROG52] and recurrent [HROG63] GBM in one case) were exposed to 2x72 h treatment. Heterologously expressed and purified *S. pyogenes* ADI was applied at 35 mU/ml, antineoplastic test substances Sorafenib, Valproate, Quinacrine, Curcumin or Resveratrol were applied in doses below IC_{30} values. Treatment effects were determined by crystal violet staining, flow cytometry cell cycle analysis and acridine orange based staining for detecting autophagy. Finally, the effect on radiosensitivity was studied.
Results: Combinations of ADI with Sorafenib, Valproate or Quinacrine boosted ADI-mediated inhibition in all tested GBM cell lines (up to 75 % killing). Of note, an individual response profile was seen for HROG52 and HROG63, with the latter showing high sensitivity towards ADI in monotherapy or in combination with Curcumin but lower reactivity when ADI was combined with Resveratrol. Acridine orange immunofluorescence stainings indicate autophagy as the main cause of tumor growth inhibition, while apoptosis and necrosis were found to play a minor role. Cell cycle arrest, detected by increased levels of cells in the S-phase, was evident following ADI and Quinacrine combination therapy. Additionally, a radiosensitizing effect was seen in one case upon ADI-based treatment in combination with Sorafenib or Valproate.
Conclusion: The cumulative in vitro results proved ADI-based therapies as a very promising regimen, especially for transferring adjuvant GBM multimodal treatments into (pre-)clinical application.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

424/FTP

Heterogeneity in dual flagellar systems

A. Pecina*[1], K. Thormann[1]
[1]*Justus Liebig Universität, Institut für Mikro- und Molekularbiologie, Gießen, Germany*

The flagellum, a helical propeller responsible for bacterial swimming and swarming, provides one of the most widespread means of bacterial motility. Although this is known since years many regulatory aspects of the flagellum and its expression and assembly still remain elusive. *Shewanella putrefaciens* possesses two distinct flagellar systems encoded by two gene clusters enabling us to study the specificity of flagellar proteins. The polar flagellum is responsible for the main propulsion of the cell and responds to the chemotaxis system, causing a temporary switch of flagellar rotation biased by gradients of attractants or repellents. The secondary, lateral flagellar system can improve spreading efficiency by increasing directional persistence. During planktonic growth in complex media, these lateral flagella are only established in a subpopulation of cells, while expression is repressed in minimal medium. This enables *S. putrefaciens* to employ a bet-hedging strategy with respect to motility: while a subpopulation of the cells saves the energy required for synthesis and maintenance of the additional flagellum, another subpopulation is motile by use of both flagellar systems to occupy new environments. However, the mechanisms regulating the formation and operation of the lateral flagellum are unknown.
We were able to show that the lateral system assembles and functions independently of the polar flagellar system and its chemotaxis system. The chemotaxis response regulator, CheY, and the regulator responsible for the correct number and assembly of the polar flagellum, FlhG, require binding to the motor switch protein FliM1 for proper function. The binding motif is absent in the lateral motor switch protein FliM2 which prevents binding of FlhG or CheY. Thus, the lateral system rotates exclusively in a counterclockwise direction and does not form a bundle with the polar flagellum.
While assembly and operation of the lateral system appear to occur in an autonomous fashion, we observed a regulatory interplay between the polar and the lateral system on a transcriptional level. The lateral flagellar gene expression seems to rely on external activators, which respond to environmental cues. We identified several proteins involved in cyclic-di-GMP signaling, which might also effect lateral flagellar gene expression and function. This second messenger is known to negatively regulate motility. The identified factors might be capable of direct sensing extracellular nutrients and modulating the intracellular c-di-GMP level in response to the nutrient situation, which in turn activates the expression of the lateral flagellar genes. The presence of such complex regulative circuits can lead to the development of heterogeneous populations.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

425/FTP

Innovative Composite Shoe components for Diabetics

M. Würtz*[1], A. Burkhardt-Karrenbrock[2]
[1]*Prüf- und Forschungsinstitut Pirmasens e.V., Pirmasens, Germany*
[2]*Colortex GmbH, Pirmasens, Germany*

The alarming increase in the number of diabetes patients means that there is a growing need for appropriate medical aids, not only in Europe but world-wide. The provision of insoles for diabetics generally pays due attention to physical and design aspects, so do requirements for other shoe components. In contrast, little attention is devoted to aspects of hygiene in spite of their enormous relevance. Diabetics are particularly susceptible to infection and frequently suffer from seriously impaired wound healing. Clothing, including shoes, have to be cleaned after wearing and disinfected where appropriate, raising the hygienic demands.
The objective of the projects were the market-oriented development of
1. an innovative composite insole system and
2. an innovative composite 3D - shaft inner lining for diabetic shoes that can be appropriately constructed for individual patients by an orthopaedic shoemaker.
Microbiological-hygienic laboratory studies by simulating the skin flora of diabetics with bacteria, yeasts, and dermatophytes at *PFI* were of crucial importance for constructive and design aspects by *Colortex* and led to improved properties of both developed composite systems with novel textile surfaces, lamination, new layered structures and a special membrane, preventing secretions and microbes from entering deeper layers.
In addition cleaning procedures were developed to ensure adequate reduction of all relevant types of germs with no alteration of shape and function of the items.

Studies performed on test persons under medical and orthopaedic supervision with skin-sensory tests and pressure measurements helped to optimise the composite shoe components and their cleaning/ disinfection procedure.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

426/FTP

Human hemofiltrate – a suitable source for antimicrobial peptides against *Clostridium difficile*?

C. Esser*[1], S. Ludger[2], A. Rodriguez[3], B. Schiel-Bengelsdorf[1], S. Weitz[1], P. Dürre[1]
[1]*Universität Ulm, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany*
[2]*Universität Ulm, Ulm, Germany*
[3]*Hannover medical school, Hannover, Germany*

Introduction: In the last decades, the number of infections with multiresistant bacteria such as *Clostridium difficile* has risen. *C. difficile* is an important pathogen and due to its antibiotic resistances the main reason of infectious diarrhea among patients in hospitals. As a result of antibiotic treatment, the normal human microflora, especially the gut microflora, is affected detrimentally. Thus, *C. difficile*, which is also able to form endospores, can flourish and colonize the gut. Colonization of the gut with *C. difficile* leads to CDAD (*C. difficile*-associated disease), which is associated with severe diarrhea and inflammation of the large intestine.
Objectives: Due to a quick development of antibiotic resistances, the useful lifespan of antibiotics is limited. Therefore, new sources for antimicrobial agents have to be found. Naturally occurring antimicrobial peptides (AMPs) are an alternative to antibiotics leading to an emerging amount of newly generated peptide banks derived from different natural sources. In this work, naturally occuring AMPs are the main focus. A peptide bank derived from human hemofiltrate was tested for growth inhibition on *C. difficile*. The fact that nearly all peptides of the human body are present in blood makes human hemofitrate a promising source for AMPs.
Material and methods: This peptide bank from human hemofiltrate was generated from ultrafiltered blood. Because of the large amount of blood needed, blood from patients suffering from renal failure and depending on dialysis had to be used. After several cation exchange and washing steps, elution using different buffers with increasing pH was performed resulting in eight pools, which were fractionated in 48 fine fractions, respectively. The resulting fine fractions were tested for growth inhibiting effect on *C.difficile*. After detecting significant growth inhibition, several purification and screening steps followed. Subsequent MS analysis then allowed identification of peptides present in this fraction.
Results: Because of significant growth inhibiting effect, one fine fraction was chosen for further analysis. Consequently, purification steps and MS analysis were done, leading to the identification of four peptides. The synthetically produced peptides were tested for growth inhibition, but all peptides did not show growth inhibition. Because MS analysis did not allow the identification of all peptides present in this fraction, further analysis was conducted. Because of the uncommon MS pattern observed, comparison to vancomycin MS pattern led to the finding that the growth inhibiting effect in this fraction was caused by vancomycin.
Conclusion: Due to the presence of vancomycin and possibly other peptide antibiotics, this peptide bank from human hemofiltrate is not suitable as a source for AMPs. A peptide bank generated from healthy donors might be more suitable for the search of AMPs against *C. difficile* and other pathogens.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

427/FTP

"CREDIBLE" – A *Cre*-med*i*ated dou*ble* reporter system to study penetration of physiological barriers by cell-penetrating effector proteins

M. L. Lubos*[1], T. Danyukova[1], B. Skryabin[2], C. Geyer[3], M. A. Schmidt[1], C. Rüter[1]
[1]*Institute of Infectiology, Center for Molecular Biology of Inflammation (ZMBE), Münster, Germany*
[2]*Core Facility TRAnsgenic animal and genetic engineering Models (TRAM), Medical Faculty, Münster, Germany*
[3]*IZKF Core Unit Preclinical Imaging eXperts PIX , Department of Clinical Radiology, Münster, Germany*

Bacterial pathogens including *Yersinia* and *Salmonella* express effector proteins that are translocated into the host cell via a type three secretion system (T3SS) during infection. Previous studies identified the effector protein YopM of *Yersinia enterocolitica* as a novel bacterial cell-penetrating effector (CPE) that is able to translocate across the host cell plasma membrane independently of *Yersinia*"s T3SS. The two N- terminal α-helices (2aH) have been identified as the protein transduction domain (PTD) of YopM, which mediates autonomous translocation and has the ability to intracellularly deliver molecular cargos such as GFP.
In order to further study and characterise the mechanisms of cell penetration by CPEs and further cell-penetrating peptides (CPPs) *in vivo*, we have generated a Cre-mediated double reporter ("CREDIBLE") system. Transgenic mice harbouring the "CREDIBLE" construct, express two reporter genes, namely near-infrared fluorescent protein (iRFP) and luciferase upon Cre/loxP-recombination.
The "CREDIBLE" system has been proven to be functional and both reporters are expressed upon recombination *in vitro*. Furthermore, crossing transgenic mice with PGK-Cre mice, expressing Cre-recombinase lead to recombination events, indicating the functionality of the system *in vivo*.
In order to analyse the distribution of CPP/CPEs *in vivo*, we have constructed different PTD-Cre fusion proteins, including 2aH-Cre. The recombinant proteins will be administered via different routes into the transgenic mice and their distribution can be analysed in real time by non-invasive live optical imaging.
In addition, the system described here can be applied in a variety of studies using drug delivery systems such as exosomes or targeted drug delivery in order to characterise their efficacy and distribution *in vivo*. Furthermore, the "CREDIBLE" system can be used to monitor bacterial or viral infections *in vivo* and, in particular, to gain a more detailed insight into the role and function of various virulence factors during infection (e.g. secreted bacterial effector proteins, outer membrane vesicles).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

428/FTP

Development of a toolkit for genetic manipulation of the acetogenic bacterium *Clostridium aceticum*

M. Karl*[1], P. Dürre[1]
[1]*Universität Ulm, Institute für Mikrobiologie und Biotechnologie, Ulm, Germany*

Introduction: *Clostridium aceticum* was the first isolated autotrophic acetogen. It is able to use gases such as syngas and H2+CO2 via the Wood-Ljungdahl pathway and forms acetate. Therefore, *C. aceticum* is an interesting biocatalyst for production of biofuels and biochemicals. Like *C. ljungdahlii* and *Acetobacterium woodii* it uses an Rnf complex to pump cations for energy conservation. Unlike those two organisms, *C. aceticum* also contains a cytochrome.

Objectives: The role of cytochromes during autotrophic growth is unclear. To elucidate the function of these cytochromes a toolkit for genetic engineering was developed.
Materials & methods: After sequencing the whole genome of *C. aceticum*, several restriction endonucleases were annotated. To test whether those endonucleases degrade the plasmid to be transformed, a restriction assay with cell free extract of *C. aceticum* was performed. Furthermore, a shuttle-vector without *Mva*I recognition sites was designed and transformed, trying different electroporation protocols.
Results: The restriction assay revealed a distinct digestion pattern, which could be associated with the *Mva*I/*Bcn*I endonuclease family protein (CACET_c27460). Therefore, the plasmid pMK83 without *Mva*I recognition sites was constructed and showed no more degradation during the restriction assay. This plasmid was then successfully transformed into *C. aceticum* using electroporation.
Conclusion: The major obstacle for successful electroporation was identified through a restriction assay. With the constructed plasmid pMK83 the restriction-/modification-system of *C. aceticum* could be overcome, which allowed us to find a suitable protocol for genetic manipulation of this organism. So far, this is the first reported, stable and reproducible transformation protocol for *C. aceticum* and is the basis for elucidating the role of cytochromes in this organism.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

429/FTP
Anaerobic degradation of aniline by *Desulfatiglans anilini* DSM4660
X. Xie*[1,2], N. Mueller[1], B. Schink[1]
[1]*Universität Konstanz, Biology, Konstanz, Germany*
[2]*Konstanz Research School Chemical Biology, Konstanz, Germany*

Aniline is widely used in the production of many industrial products, and it can cause severe pollution problems if discharged into natural waters and soils. Aniline degradation by anaerobic bacteria can play an important role in remediating the pollution. *Desulfatiglans anilini* is the only bacterium known for degrading aniline under completely anaerobic sulfate reducing conditions. However, growth of *D. anilini* on aniline is rather slow, which limits its practical application on environmental renovation and further studies on the metabolism of aniline in this strain. In our study, *Thiocapsa roseopersicina*, a purple sulfur bacterium capable of photo lithoautotrophic growth under anoxic condition in the light with sulfide as electron donor was co-cultured with *D. anilini* to stimulate the growth of *D. anilini*. Meanwhile, the activation mechanism of aniline in *D. anilini* was investigated by performing some enzymatic tests experiments. The results showed that aniline can be degraded either with CO_2 or CO as co-substrates, and CO was proved to be a far better co-substrate for aniline activation than CO_2 in the cell free extracts. Thus the sulfate reducer *D. anilini* seems to prefer a carbonylation reaction, which is energetically more favorable. The following experiments were performed by checking the enzyme activity during the conversion of aniline with CO. The activity can be measured with NAD^+ as the electron acceptor in the presence of CO and ATP, indicating that aniline should be initially carbonylated to 4-aminobenzaldehyde, which is converted to 4-aminobenzoyl CoA via 4-aminobenzoate.

Reference
[1] Schnell, S. and B. Schink, Anaerobic aniline degradation via reductive deamination of 4-aminobenzoyl-CoA in *Desulfobacterium-anilini*. Archives of Microbiology, 1991. 155(2): p. 183-190.
[2] Schmeling S, Narmandakh A, Schmitt O, Gad'on N, Schuhle K, Fuchs G. 2004. Phenylphosphate synthase: a new phosphotransferase catalyzing the first step in anaerobic phenol metabolism in *Thauera aromatica*. J. Bacteriol. 186:8044-8057.
[3] Gutierrez Acosta, O.B., N. Hardt, and B. Schink, Carbonylation as a key reaction in anaerobic acetone activation by *Desulfococcus biacutus*. Appl Environ Microbiol, 2013. 79(20): p. 6228-35.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

430/FTP
Acetate activation in *Thermacetogenium phaeum* during syntrophic acetate oxidation
A. Keller*[1], N. Müller[1], B. Schink[1]
[1]*University of Konstanz, Konstanz Research School Chemical Biology, Konstanz, Germany*

Thermacetogenium phaeum is an anaerobic, Gram-positive thermophilic bacterium that can grow with a variety of substrates. The most interesting one is acetate which *T. phaeum* converts to H2 and CO2 in a syntrophic association with a hydrogenotrophic partner organism, *Methanothermobacter thermoautotrophicus*. The Gibbs free energy gained by this complete acetate oxidation reaction is -36 kJ per mol acetate, which has to be shared by the two partner organisms. It is known that acetate is oxidized through the Wood-Ljungdahl pathway, which is also the general pathway of acetate formation in homoacetogens. Apparently this pathway can operate in both directions in the same organism. The energy-conserving mechanism in this metabolic pathway has not been elucidated yet. One ATP needs to be invested in the acetate activation to acetyl-CoA, and one ATP is gained by the conversion of formyl-tetrahydrofolate to formate. Thus, no net ATP is gained by substrate level phosphorylation in the central Wood-Ljungdahl pathway. Several energy-conserving systems have been discussed for acetogens in the literature: Rnf-complex, NADH:acceptor oxidoreductase, Ech hydrogenase or comproportionating hydrogenase. However, none of them appears to be present in *T. phaeum*. A new hypothesis was developed based on the assumption that ethanol can be produced by acetogenic bacteria including acetate activation via reduction to acetaldehyde. If in *T. phaeum* acetate could first be reduced to acetaldehyde and then be activated to acetyl-CoA nearly one net ATP could be gained. In order to confirm this hypothesis we performed enzyme assays and proteomic studies.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Free Topics (FT)
06 March 2017 • 16:00 – 18:00

431/FTP
Characterization of ExeM, an active extracellular nuclease required for biofilm formation of *Shewanella oneidensis* MR-1
M. Kreienbaum*[1], L. Binnenkade[1], K. Thormann[1]
[1]*Universität Gießen, Gießen, Germany*

Biofilms are the predominant lifestyle among all bacteria. While providing cells with an increased ability to withstand physical stress as well as antibiotics and starving conditions, it may also increase virulence of many pathogenic species. Extracellular DNA (eDNA) is a ubiquitous component of bacterial biofilms serving as an important structural part of the matrix. How eDNA is

produced, modulated and degraded (e.g. to release cells from the biofilm) is still sought to be understood.
It has been shown that extracellular nucleases are able to degrade eDNA in both planktonic cultures and bacterial biofilms to induce biofilm dispersal, for structural modulation of the biofilm matrix, utilization of DNA as nutrient source, control of horizontal gene transfer, and escape from neutrophil extracellular traps. We have previously demonstrated that the extracellular nuclease ExeM (SO_1066) is a major contributor for biofilm formation of *Shewanella oneidensis* MR-1, as the deletion of *exeM* resulted in large amounts of accumulated eDNA under biofilm conditions. Furthermore, addition of purified MBP-ExeM resulted in inhibition of biofilm formation.
Here, we further explore the characteristics of ExeM with respect to function and ability to influence biofilm formation in *S. oneidensis* MR-1. We performed in vivo and in vitro studies on various mutant variants of the protein and determined activity and co-factor requirements. In addition, the results strongly indicate that at least a significant amount of the protein localizes to the inner membrane due to a C-terminal membrane anchor which may require further processing by a rhombosortase for effective further transport and release. The study provides first more in-depth insights into the activity and transport of this well-conserved nuclease.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

432/FTP
Discovery of a species-spanning family of bacteria-derived cell-penetrating effector proteins
S. Norkowski*[1], M. L. Lubos[1], M. A. Schmidt[1], C. Rüter[1]
[1]*Institute of Infectiology, Münster, Germany*

Introduction: Pathogenic bacteria have developed intriguing strategies to hijack host immune responses using different types of virulence factors. These include effector proteins which are delivered into the host cell cytoplasm by bacterial secretion machineries such as the type III secretion system (T3SS). Previously we could identify the YopM protein of *Yersinia enterocolitica* as well as the *Salmonella* effector SspH1 as the first bacteria-derived cell-penetrating effector proteins (CPEs) that are able to translocate into host cells independent of the T3SS. Both proteins remain functional upon autonomous internalization and efficiently downregulate the expression of several pro-inflammatory cytokines. Both YopM and SspH1 belong to bacterial effectors of the LPX subtype of leucine-rich repeat (LRR) proteins which further comprise different IpaH proteins of *Shigella* as well as the *Salmonella* proteins SspH2 and SlrP.
Objectives: Due to significant homology in sequence and structure we tested the hypothesis of a general concept for T3SS-independent uptake of bacterial effector proteins of the LPX subtype.
Material and methods: The ability of recombinantly expressed LPX effector proteins to autonomously translocate into eukaryotic cells was investigated using different approaches including cell fractionation, immunofluorescence microscopy and FACS analyses. Functionality of the recombinant proteins as well as the interaction with target proteins were assessed by *in vitro* and *in vivo* assays.
Results: Indeed, we could confirm that several recombinant LPX effectors are able to enter eukaryotic cells in a T3SS-independent manner. The functionality of *Shigella*- and *Salmonella*-derived LPX effector proteins as ubiquitin E3 ligases was proven by *in vitro* ubiquitination assays. The interaction with target proteins such as NEMO could be confirmed. Moreover, the *in vivo* functionality is part of current research.
Conclusion: This study provides further evidence of a general concept of T3SS-independent translocation of bacterial LPX effector proteins. Along with their capacity to modulate and suppress host immune signaling pathways, e.g. by ubiquitination of cellular targets, bacterial LPX effector proteins represent a huge arsenal of natural modulators of inflammation and might serve as potential self-delivering biological therapeutics in the future. Moreover, the fusion to homing peptides in the sense of LEGO-like biology might allow a targeted therapy with enhanced efficacy.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

433/FTP
New laccases from *Bacillus* species: Evaluation of secretory production
S. Jansen*[1], S. Jossek[1], J. Bongaerts[1], S. Petra[1]
[1]*FH Aachen, AG Industrielle Mikrobiologie, Jülich, Germany*

Laccases belong to the multi-copper oxidases. Enzymes of this class catalyze the one electron oxidation of four equivalents of a reducing substrate coupled with the reduction of one dioxygen molecule to water. Based on the wide spectrum of reactions, which can be catalyzed by laccases, these enzymes stand out as industrial relevant enzymes, e.g. for the textile, paper and food industries as well in bioremediation and biocatalysis. While, so far, technically applied laccases are exclusively of fungal origin several bacterial enzymes have been investigated in the last years [1]. Especially, the CotA-laccases found in *Bacillus* species show interesting features for industrial applications. Compared to fungal laccases they may offer higher stability and activity in applications when higher temperature and neutral to alkaline pH is necessary [2].
The aim of this study is to characterize promising laccases from diverse strains of the genus Bacillus and closely related genera, e.g. Geobacillus. Corresponding genes were first cloned in a modified pET-based vector by Golden Gate cloning. Heterologous expression of the laccases in *E. coli* BL21 (DE3) was investigated in shake flasks and in bioreactors up to 2 L scale. In addition, according to Guan *et al*. [3], the secretory production of laccases in *Bacillus subtilis* was evaluated. Different signal peptides to direct efficient secretion of CotA-laccases were screened. Especially the use of *Bacillus subtilis* as well-established host for secretory protein production offers great potential to provide laccases in sufficient amounts for technical applications.

References
[1] Santhanam N., Vivanco J.M., Decker S.R., Reardon K.F. (2011) Trends Biotechnol., 29, 480
[2] Ihssen J., Reiss R., Luchsinger R., Thöny-Meyer L., Richter M. (2015) Sci. Rep., 5:10465
[3] Guan Z.B., Shui Y., Song C.M., Zhang N., Cai Y.J., Liao X.R. (2015) Environ. Sci. Pollut. Res. Int., 22, 9515

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

434/FTP
Phenotypical effects in *Danio rerio* by the novel heme chaperon RSAD1
K. Müller*[1], D. Jahn[1], M. Jahn[1], S. Karrie[1], V. Haskamp[1]
[1]*TU Braunschweig, Institute of Microbiology, Braunschweig, Germany*

Heme is an essential cofactor in many biological processes. However, free heme is cytotoxic and causes severe cell damage which requires a heme transporter. The bacterial protein HemW is a putative heme chaperone, which contains an iron-sulfur cluster required for dimerization and heme transfer to nitrate reductase. In recent studies *hemW* knock-out mutants in *Escherichia coli* and *Arabidopsis thaliana* showed no phenotype. RSAD1 is the homologues protein in eukarya. *In situ* hybridization analysis

revealed the expression of the *rsad1*-gene of the zebrafish *Danio rerio* in accumulating RSAD1 in head, heart and gills during the embryonic development. In addition, RSAD1 is located in the mitochondria of the cells. However, preliminary results of a *rsad1* knock-out mutant in *D. rerio* via the CRISPR/Cas9 system indicated severe anatomical abnormalities compared to a wildtype control. Thus, we aim to create a homozygous knock-out *rsad1* mutant in *D. rerio* to investigate the function and nature of the novel heme chaperone *in vivo*.
For the construction of this mutant the CRISPR/Cas9 system is used. The mix injected in *D. rerio* embryos during single-cell stadium is composed of recombinant Cas9 from *Streptococcus pyogenes*, single-giudeRNA (sgRNA) and a donor-plasmid containing *rsad1* flanking complementary sequences to allow homologous recombination. For performance enhancement of the homologous recombination and repression of non-homologues end joining the chemical compounds RS-1, Nu7026 and SCR7 were analyzed at different concentrations. Additionally, the protein Cas9 with a nuclear localization sequence at the C-terminus was recombinantly produced in BL21 *E. coli* cells and afterwards purified via a tag-chromatography. This allows the direct Cas9 injection and an immediate start of the recombination process.

Figure 1: Injection of sgRNA_*rsad1_2* in *Danio rerio* generates a phenotype
Figure 1

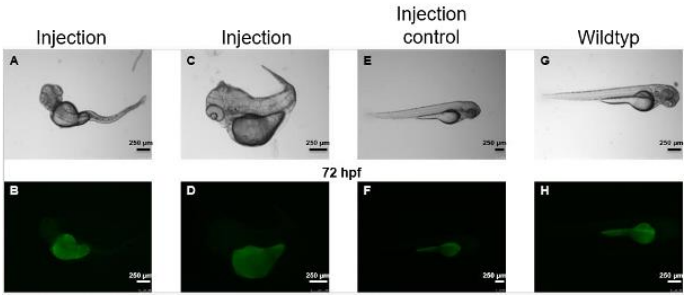
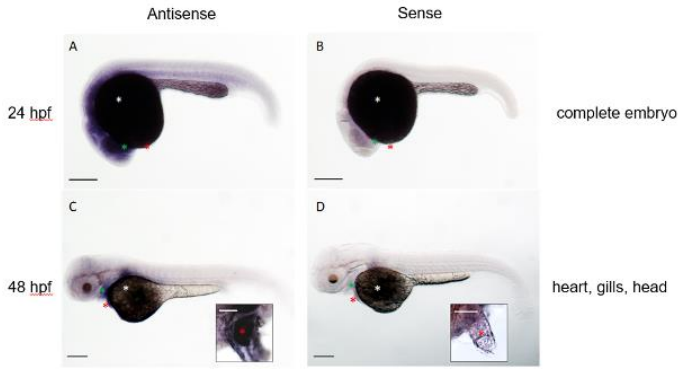


Figure 2: In situ hybridization of *rsad1* in *Danio rerio*
Figure 2



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

435/FTP
A new sactipeptide produced by *Staphylococcus spec.*
K. Kehl*[1], M. Müllender[1], C. Szekat[1], M. Josten[1], G. Bierbaum[1]
[1]*Universitätsklinikum Bonn, Institut für Medizinische Mikrobiologie, Immunologie und Parasitologie, Bonn, Germany*

Introduction: The worldwide increase and rapid spread of resistance mechanisms among bacteria require the development of new antibiotics [1]. The search for antimicrobial active substances is greatly facilitated by high-quality sequencing, assembly, and annotation techniques as well as the online availability of advanced bioinformatic tools.
Objectives: The objective of this study was the investigation of a putative novel substance produced by a clinical *Staphylococcus* isolate which had shown antimicrobial activity in previous experiments [2].
Materials & Methods: The genome of the strain was sequenced by Illumina sequencing-by-synthesis technology, assembled with SOAPdenovo, resequenced by Pacific BioSciences real-time sequencing and assembled with CLC genomics workbench, SSPACE LongRead and GapFiller. Remaining gaps were closed with PCR and sequencing. The genome was then analyzed for antibiotic gene clusters with bioinformatic tools.
Results: antiSMASH revealed a precursor and a radical SAM enzyme as part of a sactipeptide gene cluster. Sactipeptides are ribosomally synthesized and posttranslationally modified bacteriocins, specified by intramolecular thioether bridges. Their unique feature is the crosslink between the α-carbon atom of an amino acid and the sulphur of a cystein residue. The formation of these bonds is catalyzed by radical SAM enzymes and essential for the maturation of the precursor [3,4]. Additional data obtained by PCR and inverse PCR showed that the gene cluster also contains two proteases, a transcriptional regulator and an ABC transporter.
MALDI-TOF analysis affirmed the existence of a small peptide of the size of approximately 3016 Da. A disruption of the radical SAM enzyme via either homologous recombination or antisense technique was attempted to analyze the effect of the presumably resulting sactipeptide loss on antimicrobial activity and MALDI-TOF analysis. A transformation protocol was established.
Conclusion: A new sactipeptide biosynthesis gene cluster and its components were identified via genomic data analysis of a recently sequenced clinical *Staphylococcus* isolate, PCR, inverse PCR, and sequencing. The size of the peptide is estimated at about 3016 Da. Future experiments will center on the investigation of the sactipeptide regarding its structure, biosynthesis, and mode of action.

References
[1] www.who.int/mediacentre/factsheets/fs194/en/
[2] Gerson, S. (2014). Purification and characterization of novel antimicrobial substances. Unpublished.
[3] Flühe, L. et al. (2012). The radical SAM enzyme AlbA catalyzes thioether bond formation in subtilosin A. nature chemical biology; doi: 10.1038/NChemBio.798.
[4] Flühe, L., Mahariel, Mohamad A (2013). Radical S-adenosylmethionine enzyme catalyzed thioether bond formation in sactipeptide biosynthesis. Current Opinion in Chemical Biology 17(4), 605-612.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

436/FTP

Biofilm formation of *Clostridium acetobutylicum* is enhanced by oxidative stress

F. Poosch*[1], H. Bahl[1], K. Kriebel[1]
[1]*Institut für Biowissenschaften, Universität Rostock, Abteilung Mikrobiologie, Rostock, Germany*

Biofilm formation is a natural process of bacteria which can enhance the tolerance to adverse environmental stressors. Thus, also the strictly anaerobic bacterium *Clostridium acetobutylicum* might take advantage by attaching to surfaces and formatting cell layers for tolerating oxidative stress or high product concentrations in acetone-butanol-ethanol (ABE) fermentation. Established biofilms are often noticed during prolonged cultivation of *C. acetobutylicum* in a chemostat. Also, the formation of considerable amounts of a slime containing, e.g., N-Acetyl-D-glucosamine (NADG) can occasionally be observed during the fermentation process. The aim of this study was to analyze the biofilm formation of *C. acetobutylicum* in the presence of oxygen.

Wild type and the aerotolerant Δ*perR* mutant strain (Hillmann *et al.*, 2008) were cultured in clostridial growth media in uncoated or NADG coated 24-well plates. Furthermore, the cultivation was performed under anaerobic conditions and in an atmosphere containing 2 % O_2 (v/v). Biofilm formation was quantified by crystal violet staining.

The measurement of biofilm behavior for *C. acetobutylicum* was established. The results showed that the addition of NADG or the application of oxidative stress influenced the biofilm formation only slightly. However, a significant additive effect was observed when *C. acetobutylicum* was exposed to NADG and oxygen simultaneously. In addition, oxygen stress influenced the fermentation profile with increased concentrations of acetone and butanol. In contrast, the Δ*perR* mutant which has a robust oxidative stress defense showed a significant reduction of biofilm formation and no enhanced acetone and butanol production in the presence of oxygen.

Oxygen and NADG trigger biofilm formation of *C. acetobutylicum*. The oxidative stress regulator PerR seems additionally be involved in biofilm formation under oxidative stress conditions.

References

[1] Hillmann, F., Fischer, R.-J., Saint-Prix, F., Girbal, L. & Bahl, H. PerR acts as a switch for oxygen tolerance in the strict anaerobe *Clostridium acetobutylicum*. *Molecular Microbiology* 68**,** 848–860 (2008).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

437/FTP

Novel pathway for fermentation of 2,3-dihydroxypropane-1-sulfonate (DHPS) to acetate and hydrogen sulfide in *Desulfovibrio* sp. strain DHPS1

A. Burrichter*[1], K. Denger[1], D. Schleheck[1]
[1]*Universität Konstanz, FB Biologie, Konstanz, Germany*

The C3-organosulfonate 2,3-dihydroxypropane-1-sulfonate (DHPS) is one of the degradation intermediates of the widespread plant sugar sulfoquinovose (6-deoxy-6-sulfoglucose) [1, 2]. It is thus a relevant component of the biogeochemical carbon and sulfur cycles in many different environments, such as in soils, freshwater and marine systems, and in the gut of all herbivores and omnivores [1-3]. The utilization of DHPS under anoxic conditions by our new, genome-sequenced isolate *Desulfovibrio* sp. strain DHPS1, leads to a release of the sulfonate moiety as, ultimately, hydrogen sulfide, while the carbon moiety is oxidized and excreted in form of acetate. Hence, this DHPS fermentation pathway in strain DHPS1 is representative of a novel route of sulfide formation also in the human gut, where sulfide can be involved in various inflammatory diseases [4, 5]. Currently, we are identifying the enzymes involved in DHPS utilization by two-dimensional gel electrophoresis and by total proteomic analysis, when comparing cells grown under DHPS-fermenting and sulfate-respiring conditions, and our preliminary results will be presented here: It seems as if DHPS is oxidized to 3-sulfolactate by a set of dehydrogenases, and that 3-sulfolactate is then desulfonated by the enzyme complex SuyAB, yielding pyruvate for energy conservation and growth, and sulfite as electron acceptor.

References

[1] Denger, K., et al., *Sulfoquinovose degraded by pure cultures of bacteria with release of C3-organosulfonates: complete degradation in two-member communities.* FEMS Microbiol Lett, 2012. 328(1): p. 39-45.
[2] Denger, K., et al., *Sulphoglycolysis in Escherichia coli K-12 closes a gap in the biogeochemical sulphur cycle.* Nature, 2014. 507(7490): p. 114-7.
[3] Durham, B.P., et al., *Cryptic carbon and sulfur cycling between surface ocean plankton.* Proceedings of the National Academy of Sciences, 2015. 112(2): p. 453-457.
[4] Ijssennagger, N., et al., *Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon.* Proc Natl Acad Sci U S A, 2015. 112(32): p. 10038-43.
[5] Ijssennagger, N., R. van der Meer, and S.W.C. van Mil, *Sulfide as a Mucus Barrier-Breaker in Inflammatory Bowel Disease?* Trends in Molecular Medicine, 2016. 22(3): p. 190-199.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

438/FTP

Introducing PhyreBug - a novel genome browser for protein evaluation based on predicted structural homology by Phyre2

J. Poppe*[1], J. Reichelt[1], W. Blankenfeldt[1]
[1]*Helmholtz Centre for Infection Research, SFPR, Braunschweig, Germany*

In an age where antibiotic resistance among pathogens is becoming a global threat, discovery of new antibiotics and their way of action has become very important. Strong aids in this research have been protein structures. Interpreting their topology, protein functions could be assigned on a molecular level and drugs could be designed by a more rational approach. Nevertheless, discovery of novel protein folds has almost come to a halt since 2009 (PDB statistics [1]). Since drugs usually bind in protein pockets or interfaces, this could prove as a limit when it comes to designing compounds with completely new features. Our research focus is therefore to find proteins which cannot be predicted by structure prediction tools, because they are promising candidates for containing novel folds. The challenge of this approach is to find these unpredictable proteins, therefore we established PhyreBug, a web-based browser which brings together genome information and structural predictability. The browser contains whole genome information on pathogens like *P. aeruginosa* or *C. difficile* and allows for searches on criteria like protein or operon size and, most important, structural predictability based on Phyre2 predictions [2]. Proteins that fit a query are assembled in a hit list [Figure], which shows the gene and its predictability by Phyre2, a 3D homology model and background data like number of amino acids and presence/absence of transmembrane helices (TM). All information used to create the hit list is extracted from public databases and gene bank entries. With help of PhyreBug we identified numerous proteins with low sequence homology to known ones. To keep the aim of better understanding pathogens, we limited ourselves to pharmacologically interesting proteins that are related to pathogen fitness. Therefore, initial hits of the PhyreBug search were cross-

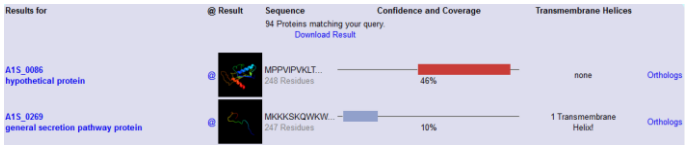
checked with published data on transposon libraries and subjected to an intensive literature search. To proof the concept only proteins matching both criteria were kept and chosen for further analysis.

Figure: *PhyreBug Hit Report. First two results of a PhyreBug search of A. baumanii ATCC_17978. Criteria: 200-300 amino acids protein size, 0-1 TM, 0-50% secondary structure predictability of the sequence. Colors show Phyre2 confidence in prediction: red-high, blue-low.*

References

[1] Berman, H.M. *et al.* (2000). The Protein Data Bank. *Nucleic Acids Research* 28(1):235-242
[2] Kelley, L.A. *et al.* (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature protocols* 10:845-858

Figure 1



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

439/FTP

Diversity of subtilisins derived from the hydrolytic phase of a biogas process and comparison to other novel subtilisins

D. Fischer*[1], L. Voss[1], S. Decker[2], I. Kuperjans[2], S. Jossek[1], J. Bongaerts[1], P. Siegert[1]
[1]*University of applied sciences Aachen, Institute for Nano- and Biotechnologie, Jülich, Germany*
[2]*University of applied sciences Aachen, Institute NOWUM-Energy, Jülich, Germany*

The ubiquitious occuring group of proteolytic enzymes find applications in various industries such as food processing, in detergents and biosynthesis [Gupta et al., 2002]. Especially extracellular proteases are of high interest. The majority of commercialized proteases are produced by bacteria, especially Bacillus spp. Since the first protease Subtilsin Carlsberg from *Bacillus licheniformis* was launched as an additive in detergents in the 1960s, numerous Bacillus-derived proteases have been characterized and probed for application. Nevertheless there is still a need for novel and improved technical enzymes. Especially biogas plant derived organisms offer a great potential of new proteolytic enzymes, due to the important role of proteases in the degradation of biomass.
After a microbial screening of samples from a biogas plant, we isolated organisms with remarkable extracellular proteolytic activity. The corresponding subtilisin genes were cloned and recombinantly expressed and secreted from *Bacillus subtilis* DB104. After fermentation of these strains the novel enzymes were purified from the supernatants via Ion Exchange Chromatography and characterized subsequently. Additionally different signal peptides with described secretory properties were tested [Degering et al.,2010].

References

[1] Gupta, R., Beg, Q.K., Lorenz, P., 2002. Bacterial alkaline proteases: molecular approaches and industrial applications. Appl. Microbiol. Biotechnol. 59, 15–32
[2] Degering C, Eggert T, Puls M, Bongaerts J, Evers S, Maurer Jaeger K. Optimization of Protease Secretion in Bacillus subtilis and Bacillus licheniformis by Screening of Homologous and Heterologous Signal Peptides. Applied and environmental Microbiology, Oct. 2010, p. 6370–6376

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

440/FTP

Biosynthesis of putative cell wall glycopolymers in *Streptomyces coelicolor* A3(2)

N. Steblau*[1], S. Sigle[1], W. Wohlleben[1], G. Muth[1]
[1]*Eberhard Karls University Tübingen, IMIT, Microbiology/ Biotechnology, Tübingen, Germany*

The Gram-positive soil bacterium *Streptomyces coelicolor* A3(2) undergoes a complex life cycle with differentiation in substrate mycelium, aerial mycelium and spores that are protected by a thick spore envelope. Sporulation septation and synthesis of the thickened spore wall are directed by the *Streptomyces* Spore Wall Synthesizing Complex[1] SSSC, a multi-protein complex including MreBCD and other proteins involved in synthesis of peptidoglycan and cell wall glycopolymers (CWG).
In this study, we aim to elucidate the role of CWGs in the life cycle of *S. coelicolor* and to characterize their biosynthesis. Two distinct CWGs, known as the Kdn-containing teichulosonic acid and polydiglycosylphosphate (PDP) have been detected in *S. coelicolor*[2,3]. The *S. coelicolor* genome encodes six putative CWG-polymerases containing a TagF-like glycerophosphotransferase domain and eleven putative CWG-transferases of the LytR-CpsA-Psr (LCP) family[4]. To identify their function, we started to delete all genes, characterized the mutant phenotypes and analyzed the cell wall composition of vegetative mycelium and spores.
Thus we could show that SCO2578 (PdtA) is a PDP-transferase, which is crucial for proper PG-incorporation at the hyphal tips under stress conditions and required to ensure the integrity of the spore envelope[4].
SCO2997 was identified as a spore wall specific PDP polymerase for the synthesis of elongated PDPs, which are involved in the proper assembly of the hydrophobic rodlet layer on the spore surfaces.

References

[1] Kleinschnitz, E.M., Heichlinger, A., Schirner, K., Winkler, J., Latus, A., Maldener, I., Wohlleben, W., and Muth, G. (2011). Proteins encoded by the mre gene cluster in *Streptomyces coelicolor* A3(2) cooperate in spore wall synthesis. Mol Microbiol. 79:1367-1379.
[2] Shashkov, A.S., Ostash, B.E., Fedorenko, V.A., Streshinskaya, G.M., Tul"skaya, E.M., Senchenkova, S.N., Baryshnikova, L.M., and Evtushenko, L.I. (2012). Novel teichulosonic acid from cell wall of *Streptomyces coelicolor* M145. Elsevier Carbohydrate Research. 359:70-75.
[3] Sigle, S., Steblau, N., Wohlleben, W., and Muth, G. (2016). A toolbox to measure changes in the cell wall glycopolymer composition during differentiation of *Streptomyces coelicolor* A3(2). J Microbiol Methods. 128:52-7.
[4] Sigle, S., Steblau, N., Wohlleben, W., and Muth, G. (2016). Polydiglycosylphosphate Transferase PdtA (SCO2578) of *Streptomyces coelicolor* A3(2) is Crucial for Proper Sporulation and Apical Tip Extension under Stress Conditions. Appl Environ Microbiol. 82:5661-72.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

441/FTP

Raw milk - a reservoir for uncommon dairy bacteriophages

E. Brinks*[1], N. Wagner[1], H. Neve[1], C. M. A. Franz[1]
[1]*Max Rubner-Institut, Microbiology and Biotechnology, Kiel, Germany*

Bacteriophages (phages) are a common threat in dairies. Phage contamination of milk fermentation processes can lead to continuous phage infections of lactic acid bacteria used as starter cultures. By doing do, phages can cause severe problemsfrom fermentation delays to total failures of fermentation batches and therefore they can cause significant financial losses. We have previously shown that phages of *Lactococcus lactis* starter strains can be remarkably thermo-resistant (1). Pasteurization of raw milk thus will not severely affect the viability of these thermo-resistant phages. Dairy phages can even survive in high numbers in whey powders after spray-drying (2). Raw milk might be a critical source for new phages, however, data on the dissemination of dairy phages in raw milk are scarce. It was reported earlier that lactococcal phages were present in 10% of raw milk samples, and phages were detected in maximal titers of up to 10^4 plaque-forming units (pfu) mL^{-1} in raw milk.
In our ongoing study, we monitored 52 raw milk samples from different dairies in northern Germany. A representative set of *Lactococcus lactis* starter culture isolates was used for phage monitoring. Notably, phages were wide-spread in raw milk and detected in 35 % of the raw milk samples. Different phage titers were determined within a wide range of $< 10^1$ to unexpectedly high numbers of $3x10^6$ pfu mL^{-1}. Electron microscopic analyses revealed that the majority of the raw milk phage population did not belong to the common group of 936-type phages usually present in dairy samples, but was related to rarely found phage types. Therefore, it can be concluded that raw milk phages may not be regarded as a major source of dairy phages which are adapted to the characteristics of industrial milk fermentation. Our study corroborates our previous DNA sequence analyses of four lactococcal raw milk phages which were also not related to the common 936 phages (3).

References

[1] Atamer, Neve, Heller, Hinrichs. 2012. Thermal resistance of bacteriophages in the dairy industry. *In*: Bacteriophages in dairy processing, pp. 195-214, Nova Publishers, Hauppauge
[2] Wagner, Brinks, Samtlebe, Hinrichs, Atamer, Kot, Franz, Neve, Heller. 2017. Whey powders are a rich source and excellent storage matrix for dairy bacteriophages. Int. J. Food Microbiol. 241, 308–317.
[3] Kot, Neve, Vogensen, Heller, Hansen, 2014. Complete genome sequences of four novel *Lactococcus* lactis phages distantly related to the rare 1706 phage species. Genome A. 2, e00265-14

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

442/FTP

Straightforward Purification of Anammox Proteins from Granular Sludge

M. Akram*[1], A. Dietl[1], T. R. M. Barends[1]
[1]*Max Planck Institute for Medical Research, Biomolecular Mechanisms, Heidelberg, Germany*

Anaerobic Ammonium Oxidation (AnAmmOx) plays a central role in the global nitrogen cycle and accounts for 50 % of the nitrogen removal from the ocean [1]. Understanding the anammox process requires the characterization of the enzymes and enzyme complexes involved in this process. Owing to their slow metabolism (doubling time 7–21 days), it takes almost 1–1.5 years to enrich anammox bacteria, which in turn hinders the supply of material to isolate proteins and protein complexes for their biochemical and biophysical characterization. Moreover, extracellular polysaccharides and other compounds make working with anammox cell lysates extremely challenging [2], which has greatly hindered biochemical and structural studies of anammox proteins. Here, we report a straightforward method for the purification of proteins from the anammox organism *Brocadia fulgida* from enriched granular sludge that enables purification of central anammox proteins on the 50 mg scale. *Brocadia fulgida* was confirmed to be the only representative anammox species in the biomass using the hydrazine synthase alpha (HZS-α) subunit as a unique molecular marker. In addition, Aerobic Ammonium-Oxidizing Bacteria (AOB), primarily *Nitrosomonas europaea* were found using hydroxylamine oxidoreductase (HAO) degenerate primers specific only for AOB. After a combination of mechanical and enzymatic lysis methods, central anammox proteins such as HZS, hydrazine dehydrogenase (HDH) and hydroxylamine oxidase (HOX) could be purified to homogeneity using anion exchange-, hydroxyapatite- and gel filtration chromatography with little or no evidence of remaining polysaccharides. The identity of the isolated proteins was confirmed by peptide mass fingerprinting. All purified proteins showed characteristic UV-Vis spectra in their as-isolated and dithionite-reduced states as well as in the presence of their substrates (hydroxylamine and hydrazine). HDH and HOX were shown to be optimally active in catalysing oxidation of hydrazine and hydroxylamine, respectively, and both proteins were pure enough for crystallization.

References

[1] K. R. Arrigo, Nature 437, 349, (2005)
[2] I. E. Cirpus et al., Int. J. Biol. Macromol. 39, 88 (2006)

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

443/FTP

Lactate – Acetate co-fermentation in *Clostridia*

N. Müller*[1], S. Röther[2], K. Porsch[2]
[1]*University of Konstanz, Biology, Konstanz, Germany*
[2]*Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany*

Several *Clostridium* strains are able to ferment lactate to butyrate and hydrogen only in the presence of acetate although the reaction without acetate would be exergonic enough to support growth. It was suggested that lactate is oxidized to pyruvate and acetyl-coenzyme A. Additionally, acetate is converted to acetyl-coenzyme A to yield enough acetyl-coenzyme A to generate acetoacetyl-coenzyme A which is then reduced to butyrate through reversed beta-oxidation. Even though the participating enzymes of the pathway are known, the initial reaction of lactate oxidation and the flow of electrons from lactate oxidation to crotonyl-coenzyme A reduction was never convincingly proven. We have reevaluated this pathway in the light of the concept of bifurcating enzyme reactions. Accordingly, lactate could be oxidized endergonically with NAD^+ driven by reduction of another molecule of NAD^+ with reduced ferredoxin by a bifurcating lactate dehydrogenase similar to the one observed earlier in *Acetobacterium woodii* (1). Reduced ferredoxin could be provided by the subsequent oxidation of pyruvate to acetyl-coenzyme A. NADH arising during this reaction could then be reoxidized to NAD^+ by a bifurcating butyryl-coenzyme A dehydrogenase similar to the one of *Clostridium kluyveri*, where NADH oxidation with crotonyl-coenzyme A drives the endergonic oxidation of another molecule of NADH with ferredoxin (2). Alternatively, two molecules of lactate could directly be oxidized with NAD^+ and crotonyl-coenzyme A without involvement of ferredoxin. To find proof for one of these two alternative hypothetical pathways, we have analyzed the proteome of several lactate – acetate cofermenting strains and assayed activities of the participating enzymes.

References
[1] Weghoff, M. C., Bertsch, J., and Müller, V. (2015) A novel mode of lactate metabolism in strictly anaerobic bacteria. Environ Microbiol, 17: 670–677. doi:10.1111/1462-2920.12493.
[2] Li, F., Hinderberger, J., Seedorf, H., Zhang, J., Buckel, W., and Thauer, R. K. (2008) Coupled Ferredoxin and Crotonyl Coenzyme A (CoA) Reduction with NADH Catalyzed by the Butyryl-CoA Dehydrogenase/Etf Complex from *Clostridium kluyveri*. J Bacteriol, 190(3):843-850.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

444/FTP

Precision-Cut Lung Slices (PCLS) as an *ex-vivo* model for respiratory infections in cattle and goats caused by *Mycoplasma* species

Y. B. Weldearegay*[1], R. Brehm[2], A. Kostka[1], N. Rüger[1], A. Schulze[1], P. Valentin-Weigand[1], J. Meens[1]
[1]*Institute for Microbiology, University of Veterinary Medicine Hannover, Department of Infectious Diseases, Hannover, Germany*
[2]*Anatomy Institute, University of Veterinary Medicine Hannover, Hannover, Germany*

Introduction: The genus Mycoplasma comprises over 130 species, all exhibiting an obligate parasitic mode of life in combination with a rather strict host and tissue specificity. In ruminants, respiratory infections caused by Mycoplasma species lead to considerable economic losses due to treatment cost, production loss and trade restriction. Important pathogens are *Mycoplasma mycoides* subsp. *mycoides* (*Mmm*), the causative agent of contagious bovine pleuropneumonia (CBPP), *Mycoplasma mycoides* subsp. *capri* (*Mmc)*, which causes pneumonia, mastitis, arthritis and septicaemia in goats and *Mycoplasma bovis* (*M. bovis*), an important pathogen associated with the bovine respiratory disease (BRD) complex. The control of these diseases mainly relies on a better understanding of host-pathogen interactions and the subsequent development of better diagnostics and vaccines. Precision cut lung slices (PCLS) are organotypic tissue models, which are widely used in lung research to study airway contraction, local immune reactions and bacterial or viral lung infections. PCLS reflect the microanatomy of the respiratory tract that comprises widely varying cell types that may respond differently to the same stimulus. Therefore, PCLS are better suited to mimic the natural host than cell lines. They can limit the number of experimental animal infections, which is desirable from the animal welfare point of view.
Objective: Establishment of a PCLS infection model to study host-pathogen interactions in different mycoplasma species. Characterization of bacterial adhesion to host cells, host specificity, cell tropism and cytotoxicity.
Materials & methods: Lungs were obtained from apparently healthy slaughtered adult cattle and goats. The accessory and cranial lobes were used for the preparation of PCLS. The viability of PCLS were monitored by cilia movement, MTS assay and live-dead staining. Slices were infected with freshly grown Mycoplasma strains for 4 h. After removal of unbound bacteria, slices were further incubated and samples preserved every 24h for a total of 4 days. Investigation of slices was done using histological staining, immunohistochemistry and fluorescent microscopy. Moreover, the infection burden was monitored using plating out of bacteria and qRT-PCR.
Results: Uninfected bovine and caprine PCLS remained viable up to 10 days with full ciliary activity. All tested Mycoplasma strains adhere to respiratory cilia. In addition, the titer of *Mmm* in caprine PCLS decreased over time, indicating species specificity of the pathogen. RNA preparation from infected PLCS was established and allowed transcriptional analysis of Mycoplasma genes during infection.
Conclusion: The PCLS system is a suitable model to study host-pathogen interaction in the tested mycoplasma species.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

445/FTP

Fermentation of an oxidized sugar by *Anaerobium acetethylicum*: evidence for the involvement of a modified Entner-Doudoroff pathway

Y. Patil*[1], M. Junghare[1], N. Mueller[1]
[1]*University of Konstanz, Dept. of Biology, Microbial Ecology, Konstanz, Germany*

Fermentation is a promising field for the production of commercial chemicals from a wide variety of organic substrates. In the past, much effort was dedicated to elucidate the pathways for utilization of hexose sugars by microorganisms, but little is known about the metabolism of oxidized hexose sugars, e.g. gluconate. Here we present the metabolic network for the utilization of gluconate by *Anaerobium acetethylicum* strain GluBS11T (Patil *et al., 2015*) isolated from an anaerobic enrichment sample from a methanogenic bioreactor. The detailed metabolic pathway for gluconate fermentation by *Anaerobium acetethylicum* strain GluBS11T was investigated by enzyme assays and proteome studies. Especially, three possible routes for the initial conversion of gluconate to pyruvate and glyceraldehyde-3-phosphate were investigated. Gluconate degradation could occur through the pentose phosphate pathway, the Entner-Doudoroff (ED) pathway or a modified ED pathway. Apparently, *Anaerobium acetethylicum* strain GluBS11T uses a modified Entner-Doudoroff pathway for the degradation of gluconate. Gluconate is converted to 2-keto-3-deoxy-gluconate (KDG) by gluconate dehydratase through a simple dehydration reaction. KDG is then converted into 2-keto-3-deoxy-6-phospho-gluconate (KDPG) by KDG kinase and KDPG is converted to pyruvate and glyceraldehyde-3-phosphate by KDPG aldolase. Further glyceraldehyde-3-phosphate is converted to pyruvate through the lower part of glycolysis. The main enzymes involved in this conversion of gluconate are gluconate dehydratase, KDG kinase and KDPG aldolase. Further, pyruvate is converted to acetate, formate, ethanol and hydrogen as main fermentation products.

References
[1] Patil, Y., Junghare, M., Pester, M., Müller, N., and Schink, B. (2015) Characterization and phylogeny of *Anaerobium acetethylicum* gen. nov., sp. nov., a strictly anaerobic gluconate-fermenting bacterium isolated from a methanogenic bioreactor. *Int J Syst Evol Microbiol* 65: 3289-3296.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

446/FTP

A simple, rapid and cost-effective process for production of latex clearing protein to produce oligopolyisoprene molecules

R. Andler*[1], A. Steinbüchel[1]
[1]*Westfälische Wilhelms-Universität Münster, Molecular Microbiology and Biotechnology, Münster, Germany*

Aiming at finding feasible alternatives for rubber waste disposal, the partial enzymatic degradation of poly(*cis*-1,4-isoprene)-containing materials represents a potential solution. The use of rubber-degrading enzymes and the biotransformation of rubber into new materials is limited by the high costs associated with the production and purification of the enzyme and the complexity of the process. This study presents a simple and low-cost procedure to obtain purified latex clearing protein from *Gordonia polyisoprenivorans* strain VH2 ($Lcp1_{VH2}$), an enzyme capable of

cleaving the double bonds of poly(*cis*-1,4-isoprene) in presence of oxygen to produce different size of oligomers with terminal aldehyde and ketone groups, respectively. The gene coding for $Lcp1_{VH2}$ was overexpressed in *Escherichia coli* C41 (DE3), and by using an auto-induction medium high protein yields were obtained. Purification of the enzyme was performed using salting out precipitation with ammonium sulfate. Different salt concentrations and pH were tested in order to find the optimal for purification. The enzymatic activity of the purified enzyme was measured by an oxygen consumption assay in the presence of polyisoprene latex. The results showed an active and partial purified fraction of $Lcp1_{VH2}$, able to cleave the backbone of poly(*cis*-1,4-isoprene) and to produce degradation products that were identified with staining methodologies (Schiff reagent for aldehyde groups and 2,4-DNPH for carbonyl groups) and characterized using nuclear magnetic resonance (NMR). Thirteen different storage conditions were tested for the purified enzyme analyzing the enzymatic activity after 1 and 3 months. $Lcp1_{VH2}$, as an ammonium sulfate precipitate, was stable, easy to handle and sufficiently active for further analysis. The described methodology offers the possibility to upscale the process and to produce large amounts of this protein.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

447/FTP

Prevalence of antibiotic-resistant water-borne pathogens in two Bavarian rivers

D. Calomfirescu*[1]
[1]*Bayer. Landesamt für Gesundheit und Lebensmittelsicherheit, Oberschleißheim, Germany*

Context: The increased prevalence-rates of multidrug-resistant bacteria in patients and animals worldwide represent a threat to human health. In this context it is considered that wastewater can act as a potential reservoir and transmission route for these bacteria. In fact, in the aquatic environment horizontal gene transfer of virulence and resistance genes can occur among bacteria, increasing the prevalence of multidrug-resistant bacteria in the receiving freshwater environments, such as rivers.
Objectives: The aim of this work was to assess the prevalence of multidrug-resistant bacteria in two Bavarian rivers by determination of the emergence of multidrug-resistant *Enterobacteriaceae,* such as *E.coli* and *Klebsiella pneumoniae,* as well as healthcare-associated nonfermenters, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and molecular characterization of their antimicrobial resistance determinants.
Methods: From April 2015 to September 2016, 240 river water samples were collected, among them 163 water samples from the river Isar at 13 distinct sites, and 77 water samples from the river Ilz at 7 different locations. After the filtration of water samples through 0.45 micrometer-pore-size membrane filters and isolation of the multidrug-resistant bacteria on selective media, these were identified and tested for antimicrobial susceptibility by BD Phoenix™ according to manufacturer"s instructions**.** The screening for the detection of antimicrobial resistance genes was performed by multiplex PCR and subsequent sequencing.
Results & Discussion:
Multidrug-resistant bacteria were found to be ubiquitous in the water samples collected. Bacteria belonging to the *Acinetobacter baumannii/ baumannii calcoaceticus complex* were most prevalent in the river Isar (14 % of the total of detected multidrug-resistant isolates, n= 55/406). In contrast, cefotaxime-resistant (CTX-R) *E.coli* isolates were the most prevalent bacteria in the river Ilz (18 % of the total of detected multidrug-resistant isolates, 34/190). Our results showed that faecal indicator bacteria *E. coli* and *intestinal Enterococci* were more abundant in the river Ilz than in the river Isar. This might be due to the fact that the river Ilz is influenced by wastewater from a high number of small and medium-sized wastewater treatment plants and runoffs from agricultural land. In contrast the catchment area of the river Isar is dominated by forests, grasslands and urban residential areas.
The preliminary results of the molecular characterization by multiplex PCR for CTX-R *E. coli* strains showed the predominance of genotype CTX-M-15 (in Isar samples 56%, 13/23; in Ilz samples 48%, 12/25), followed by CTX-M-1. Our findings are in accordance with the global spread of CTX-M15, being one of the most common ESBL-genotypes in humans and amongst others responsible for urinary tract and bloodstream infections in both community and hospital settings. Among the ESBL-producing *E. coli* isolated from river Isar the most frequently encountered phylogenetic group was group A (33 %, 10/30), followed by group D (23 %, 7/30), whereas in the river Ilz the most predominant phylogroup was B2 (33 %, 11/33), followed by phylogroup A and D to the same extent (21 %, 7/33). The distribution of phylogenetic groups in our study suggests that the prevalence of extra-intestinal pathotypes and enteropathogenic isolates, assigned to group B2, and D, respectively, was noteworthy. So far we did not detect carbapenemase resistance in *Pseudomonas aeruginosa* or *Acinetobacter baumannii.* The preliminary results are stated for 90 % of the total amount of the above mentioned isolates.
Conclusions: Taken together, our results suggest that the sampling sites of the two Bavarian rivers Ilz and Isar are a potential reservoir for multidrug-resistant bacteria and antimicrobial resistance determinants. This study highlights the need to identify potential transmission routes of these bacteria and their virulence factors from rivers into the population, e.g. via irrigation. In conclusion, our results indicate that multidrug-resistant bacteria are an emerging challenge within aquatic environments and they confirm that the development of national regulations for water used for irrigation is an important issue.
Funding: This study was supported by Bundesministerium für Bildung und Forschung (BMBF) under the grant number 02WU1346B. The authors bear sole responsibility for the content of this article.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

448/FTP

de.NBI - German Network for Bioinformatics Infrastructure

A. Al-Dilaimi[1], T. Dammann-Kalinowski[1], A. Pühler[1], F. Sprengel[1], A. Tauch[1], D. Wibberg*[1]
[1]*Bielefeld University, de. NBI Administation Office, c/o Center for Biotechnology, Bielefeld, Germany*

In recent years, the modern life sciences research underwent a rapid development that was driven mainly by the technical improvements in analytical areas in terms of miniaturization, parallelization and high through-put of biological samples and thus the generation of huge amounts of experimental data. Prominent examples of this ongoing development are the omics techniques featuring the analysis of the various levels of information storage and processes in living cells, and the numerous new imaging techniques providing insights into biological systems to a hitherto unprecedented depth. The ever growing application of these novel techniques and the exploitation of the resulting data have revolutionized many fields of science and are furthermore opening new areas of basic and applied research with considerable opportunities for life sciences. The bottleneck that prevents realization of the full potential of the different omics technologies is not the data generation itself, but the subsequent data analysis.
The German Network for Bioinformatics Infrastructure (de.NBI) takes care of this challenge in many areas of life sciences with its

mission to provide, expand and improve a repertoire of specialized bioinformatics tools, appropriate computing and storage capacities and high-quality data resources. These efforts are supplemented by a training program providing courses on the supplied tools.
de.NBI is an academic funding initiatve of the German Ministry of Research and Education (BMBF) and started in March 2015. The consortium currently consists of 40 project partners organised in eight service centers and one central administration and coordination unit. The service centers offer a variety of training courses and bioinformatics services, online databases, software libraries, and tools as webservices and/or for download. Furthermore, consulting on individual issues is available. Services are aimed at application users in life sciences as well as bioinformaticians and developers. The de.NBI services will be unified with regard to standards, interchangeability and reproducibility.
Further reading at: http://www.denbi.de

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Gastrointestinal Infections (FG GI)

449/GIP

An acid-responsive ArsZ sRNA modulates *Helicobacter pylori* urease activity at low pH through regulation of a histidine-rich nickel-binding protein.

H. S. Tan*[1], D. Beier[2], C. Sharma[1]
[1]*University of Würzburg, Research Center for Infectious Diseases (ZINF), Würzburg, Germany*
[2]*University of Würzburg, Biocenter, Würzburg, Germany*

Helicobacter pylori, a Gram-negative, pathogenic Epsilonproteobacterium, colonizes the stomachs of about 50% of the world"s population and is associated with peptic ulcers, chronic gastritis, and gastric cancer. The small *H. pylori* genome (1.67 Mb) encodes relatively few transcriptional regulators, including only three complete regulatory two-component systems (TCS) (1). Moreover, little is known about post-transcriptional regulation in *H. pylori*. Like 50% of all sequenced bacteria, the *H. pylori* genome does not encode a homolog of the RNA chaperone Hfq, a key player in small, regulatory RNA (sRNA)-mediated regulation in enterobacteria. Based on differential RNA-seq we have identified more than 60 candidate sRNAs in *H. pylori* strain 26695 (2), suggesting an uncharacterized layer of post-transcriptional riboregulation in this pathogen. During colonization of the human stomach, low pH is the main environmental stress encountered by *H. pylori*. To ensure its survival under acidic conditions, *H. pylori* utilizes urease, a nickel-activated metalloenzyme which cleaves urea into ammonia to buffer the pH in the periplasmic space (3). Expression of the urease operon is tightly regulated at the transcriptional and post-transcriptional level. Moreover, the urease activity is modulated post-translationally via the activity of nickel-binding proteins, such as the histidine-rich protein HP1432 (4). Here we show that a conserved and abundant sRNA, ArsZ (Acid Responsive sRNA Z), and its target HP1432, constitute yet another level of urease regulation. The acid-responsive ArsRS TCS (5) represses ArsZ expression at low pH. ArsRS and ArsZ sRNA work in tandem to regulate expression of HP1432 via a coherent feed-forward loop. The ArsRS TCS activates HP1432 transcription in response to low pH. *In vitro* and *in vivo* experiments show that ArsZ interacts with the ribosome binding site of HP1432 mRNA, repressing translation of HP1432. ArsZ thereby fine-tunes the dynamics of urease activity after a shift to low pH presumably by altering nickel availability through post-transcriptional control of HP1432 expression. To our knowledge, this is the first example of a *trans*-acting sRNA that regulates a nickel storage protein to modulate apo-urease maturation, and thereby might prevent overproduction of ammonia which could be detrimental to the cell at neutral pH.

References

[1] Tomb JF, White O, Kerlavage AR, Clayton RA, *et al.* (1997) The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* 388: 539-547.
[2] Sharma CM, Hoffmann S, Darfeuille F, *et al.* (2010) The primary transcriptome of the major human pathogen *Helicobacter pylori*. *Nature* 464: 250-255.
[3] Bauerfeind P, Garner R, Dunn BE & Mobley HL.(1997) Synthesis and activity of *Helicobacter pylori* urease and catalase at low pH. *Gut* 40: 25–30.
[4] Seshadri S, Benoit SL & Maier RJ (2007) Roles of His-rich hpn and hpn-like proteins in *Helicobacter pylori* nickel physiology. *J Bacteriol* 189: 4120-4126.
[5] Pflock M, Finsterer N, Joseph B, Mollenkopf H, Meyer TF & Beier D. (2006) Characterization of the ArsRS regulon of *Helicobacter pylori*, involved in acid adaptation. *J Bacteriol* 188: 3449-3462.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

450/GIP

Calprotectin and its bactericidal effect on *Vibrio cholerae*

C. Toulouse*[1], J. Krzistetzko*[1], G. Fritz[2], J. Steuber[1]
[1]*University of Hohenheim, Institute of Cellular Microbiology, Stuttgart, Germany*
[2]*University of Freiburg, Institute of Neuropathology, Freiburg, Germany*

Introduction: S100A8 and -A9 are members of the S100 protein family. In their heterodimeric form they are known as calprotectin. During inflammation calprotectin is secreted by mammalian cells such as neutrophils. Calprotectin can bind four calcium ions through the EF-hand motifs, which promotes binding of transition metals like zinc and manganese with high affinity [1]. Since those metals are essential for bacteria, we investigated the impact of calprotectin on *Vibrio cholerae*, the causative agent of the Cholera disease.
Objectives: Cholera disease is still a major health issue, since no reliable, persistent vaccine is known. Through calprotectin"s sequestration of Zn^{2+} and Mn^{2+} it may possess antimicrobial activity against *Vibrio cholerae"s* growth and virulence [2].
Materials & Methods: Human S100A8 and -A9 were overexpressed in *E. coli* BL21(DE3) cells. Harvested cells were denatured in guanidinhydrochlorid. S100A8 and -A9 were combined and refolded during dialysis. Heterodimers were purified by ion exchange chromatography and size exclusion chromatography. Growth experiments were performed with calcium containing minimal medium supplemented with Zn^{2+} and Mn^{2+}, if indicated.
Results: The S100A8 and S100A9 heterodimer was overexpressed and purified with IEC and SEC out of *E. coli* cells. Heterodimer formation was confirmed by SDS page and antimicrobial properties on the growth of *Vibrio cholerae* were observed.
Conclusion: Together with reactive oxygen species, calprotectin is released in high amounts by neutrophils during pathogenic infection and acts as component of the nutritional immunity by chelating metal ions such as zinc and manganese. *Vibrio cholerae* harbors a Mn2+ depended cytosolic- and a Cu^{2+}/Zn^{2+} dependent periplasmic superoxide dismutase [3], which protect the bacteria against oxidative damage during the respiratory burst of the host immune system. Thus Mn^{2+} and Zn^{2+} availability is critical for *V. cholerae* growth.
This work was supported by DFG grant FR1321/5-1 (to JS).

References
[1] Damo, S.M., et al., *Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens.* Proceedings of the National Academy of Sciences of the United States of America, 2013. 110(10): p. 3841-6.
[2] Liu, J.Z., et al., *Zinc sequestration by the neutrophil protein calprotectin enhances Salmonella growth in the inflamed gut.* Cell host & microbe, 2012. 11(3): p. 227-39.
[3] Gabbianelli, R., et al., *Vibrio cholerae periplasmic superoxide dismutase: isolation of the gene and overexpression of the protein.* Journal of biotechnology, 2004. 109(1-2): p. 123-30.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

451/GIP

Abstract has been withdrawn.

452/GIP
Contamination of Ready-to-Eat Salads with *Clostridium difficile*
S. Maurischat*[1], A. Hanuschik[1], H. Wichmann-Schauer[1]
[1]*Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany*

Clostridium (*C.*) *difficile* is a major cause of gastrointestinal disease, often associated with antibiotic treatment. Therefore, *C. difficile* infections (CDI) are traditionally considered to occur predominantly in a nosocomial context. But this view has been changing dramatically, since an increasing number of cases have been classified to be community-acquired in the last decade. Especially, the infection sources of these CDI cases are mostly unknown. The contact to asymptomatic human carriers as well as a zoonotic transmission might play an important role. Evidence for the latter has been provided by recent studies based on human and animal isolates with identical PCR-ribotypes, PFGE patterns or MLVA types.
To investigate a potential contamination of food products, the Federal Institute for Risk Assessment has validated a cultural and molecular *C. difficile* detection method for ground meat and salad. The results of the validation (specificity, sensitivity, accuracy, in- and exclusivity) of the detection method as well as first results regarding the contamination of approximately 200 samples of fresh-cut salad from retail will be presented. Furthermore, all isolated *C. difficile* strains will be characterized regarding their PCR-ribotype and toxigenic potential.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

453/GIP
Actives identification of small compounds for metabolic and motility inhibition of *Helicobacter pylori*
N. Coombs[1,2], D. Pscheniza[1,2], F. Banović[1,2], E. Gripp[1,2], I. Riedel[1,2], U. Bilitewski[2,3], M. Brönstrup[2,3], S. Suerbaum[1,2], C. Josenhans*[1,2]
[1]*Hannover Medical School, Institute for Medical Microbiology, Hannover, Germany*
[2]*German Center for Infection Research DZIF, Hannover-Braunschweig, Germany*
[3]*Helmholtz Center for Infection Research HZI, Department Chemical Biology, Braunschweig, Germany*

Helicobacter pylori is a notable human pathogen, infecting more than half of the global population. Due to being a causative agent of chronic gastritis, gastric ulcer and gastric cancer, it presents a major concern for medical authorities worldwide. *H. pylori* infections are chronic and do not seem to cease spontaneously without combination antibiotic therapy. Additional considerations concern damage to the microbiota, a side effect of most antibiotics. Improving the therapy and overcoming increase in the resistance to common antibiotics makes research into new therapeutic approaches paramount.
The aim of our project within the German Centre for Infection Research (DZIF) is the identification and development of novel therapeutics against *H. pylori*. Priority is given to potential antivirulence compounds, since they may cause less harm to human symbiont bacteria as well as show reduced incidence of resistance against such compounds. Our current approach is based on inhibition of motility and the motility-associated type III secretion system of *H. pylori* – no clinical isolates of *H. pylori* which had dysfunctional motility have been found so far, which indicates its importance for *H. pylori* colonization and survival within the human stomach.
We performed screens of multiple libraries containing more than 4000 compounds of both natural and synthetic origin. Hits from the primary screening assay focused on motility inhibition of *H. pylori* were further analyzed in secondary assays, characterizing their effects on vitality and measuring IC50, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). For putative target identification of selected compounds, different avenues were taken. First, expression analysis (RT-PCR) of different transcript categories was performed. Furthermore, resistant clones of *H. pylori* were generated and compared to the wild type bacteria for growth behavior and resistance to test compounds. The basis of resistance and compound targets is currently being further approached by whole genome sequencing.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

454/GIP
Horizontal gene transfer of Integrating Conjugative Element ICE*Hptfs4* in *Helicobacter pylori*
W. Fischer*[1], C. Spicher[1], E. Weiss[1], R. Haas[1]
[1]*Max von Pettenkofer-Institut, Muenchen, Germany*

Similar to the situation in many other pathogenic bacteria, individual isolates of the human gastric pathogen *Helicobacter pylori* may harbour different genome islands. The most prominent example is the *cag* pathogenicity island, which encodes a type IV secretion system and the translocated effector protein CagA, a well-characterized risk factor for cancer development. We have previously characterized two further, potentially virulence-associated, genome islands, which are often referred to as plasticity zones, but have typical features of integrating conjugative elements (ICEs). For example, they carry independent type IV secretion systems that may enable their transfer between bacterial cells, and they integrate into particular sites within the genome.
Here, we have characterized excision and transfer capabilities of one such ICE (termed ICE*Hptfs4*), using molecular genetic techniques. ICE*Hptfs4* harbours the *dupA* gene and other genes which have been associated with duodenal ulcer development. We show that recombinase-mediated excision from the chromosome uses a characteristic sequence motif to generate a circularized intermediate, and we have estimated the excision rates by taking advantage of a circularization-dependent promoter. Horizontal transfer of the ICE*Hptfs4* element from donor to recipient cells was observed after mating experiments in the presence of DNase, suggesting a conjugative mechanism. Nevertheless, examination of transfer rates is complicated by the potent natural transformation (ComB) and homologous recombination (HR) systems of *H. pylori*, which result in a high efficiency of HR-mediated exchange of ICE fragments, while transfer of the complete element seems to be very rare under laboratory conditions. Interestingly, we found that the presence of the ComB system in the recipient strain enhances, but is not essential for,

ICE gene transfer, whereas the ComEC protein as well as recombination system proteins are strictly required.
In conclusion, we have shown that *H. pylori* ICE*Hptfs4* is a mobile genetic element which can be horizontally transferred by a conjugation-like mechanism which nevertheless depends on the recipient cell transformation and recombination machinery. The impact of such transfer events and of ICE gene expression on *H. pylori* pathogenicity remains to be shown in future studies.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

455/GIP

Linkage of chitinase production by *Listeria monocytogenes* with disease outcome of listeriosis in humans

S. Halbedel*[1], S. Banerji[1], R. Prager[1], J. Rismondo[1,2], E. Trost[1], G. Alles[3], W. Ruppitsch[4], S. Kleta[5], C. Hölzel[6], F. Schlesiger[7], A. Flieger[1]
[1]*Robert Koch Institute, FG11-Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany*
[2]*Imperial College London, Section of Microbiology, London, United Kingdom*
[3]*Health Office Paderborn, Paderborn, Germany*
[4]*Austrian Agency for Health and Food Safety (AGES), Laboratory for Listeria, Vienna, Austria*
[5]*Federal Institute for Risk Assessment, Department of Biological Safety, Berlin, Germany*
[6]*Faculty of Veterinary Medicine, Chair of Milk Hygiene, Munich, Germany*
[7]*Chemical and Veterinary Analytical Institute Ostwestfalen-Lippe (CVUA-OWL), Detmold, Germany*

A cluster of 44 human *L. monocytogenes* serotype IVb isolates with identical PFGE profiles was detected by the binational German-Austrian consiliary laboratory for *L. monocytogenes* in spring 2015. All but one isolates originated from stool samples of young children (2-5 years) with non-invasive gastroenteritis. A notable exception was one clone isolated from a 72 year old patient suffering from invasive listeriosis. Epidemiological investigations identified rice pudding contaminated with high loads of *L. monocytogenes* as the most probable source of infection and isolates from this source had indeed the same PFGE type. Genome sequencing and core genome MLST using a scheme containing 1701 alleles revealed that the non-invasive isolates from young children formed a cluster together with all food isolates, whereas the isolate from the invasive listeriosis patient differed in 51 alleles. More detailed genome comparisons identified 180 sequence variations between one non-invasive and the invasive clone and these included 36 non-silent changes. Among the changes clustering with disease outcome in an extended set of strains either causing invasive or non-invasive disease was a pre-mature stop codon in the *lmo0105* gene encoding chitinase B. We demonstrate that the non-invasive *L. monocytogenes* isolates - in contrast to all tested invasive isolates - do not secrete chitinase B and are unable to degrade chitin. Chitinases have been linked with *L. monocytogenes* virulence in mouse models, but their precise role during the infection process is still unknown (1). The results presented here indicate that chitinase production could even be important during infection of humans with *L. monocytogenes* and possibly other pathogens.

References

[1] Chaudhuri *et al.* 2010. *Appl Environ Microbiol. 76:*7302-5.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

456/GIP

L-glutamine is an important mediator of growth and virulence in *Salmonella enterica*

P. Auraß[1], J. Düvel[1], S. Karste[1], U. Nübel[2], W. Rabsch[1], A. Flieger*[1]
[1]*RKI, Wernigerode, Germany*
[2]*DSMZ, Braunschweig, Germany*

We here describe a small colony variant (SCV) which spontaneously emerged from an unculturable water microcosm inoculated with *Salmonella enterica* servovar Typhimurium. We clarify the genetic basis of the SCV as frameshift mutation in the gene of glutamine synthetase GlnA presumably leading to low levels of internal L-glutamine. The internal glutamine pool serves as a sensor for external nitrogen availability and is thought to regulate cell growth which is one of the traits affected in the SCV. It is important to study the mechanisms leading to SCV formation on a molecular basis because SCVs a) may arise spontaneously upon stress exposure including environmental and host-defense stresses, b) are slow growing, show decreased susceptibility to antimicrobials, and therefore are difficult to eradicate, and c) only a few descriptions of *S. enterica* SCVs are yet available. We describe that decreased virulence of the SCV depended on the *glnA* mutation. Specifically, invasion/uptake into host cells is reduced which is caused by a depletion of SPI-1 virulence proteins and flagellin. The strain moreover rendered resistant to lysis by the *Salmonella* genus phage Felix O1 which is used in phage typing and proposed as therapeutic agent. Supplementation with L-glutamine or genetic reversion of *glnA* truncation restored growth, cell entry, and protein abundance. In summary, our study shows that a point mutation in a spontaneously emerged *S. enterica* variant attributes for a variety of phenotypic changes and identifies glutamine synthetase and accordingly internal glutamine levels as a coordinator of growth and virulence in *S. enterica*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

457/GIP

Phenotypic diversity of clinical *Clostridium difficile* isolates

D. Wetzel*[1], P. Plorin[1], O. Zimmermann[1], M. Rupnik[2], L. von Müller[3], K. Gunka[1], U. Groß[1]
[1]*University Medical Centre (UMG) Göttingen, Institute of Medical Microbiology, Göttingen, Germany*
[2]*University of Maribor, National laboratory for health, environment and food, Maribor, Slovenia*
[3]*University of Saarland, Institute of Medical Microbiology and Hygiene, Homburg/Saar, Germany*

The *C. difficile* population comprises five clonal lineages of virulent strains with significant genetic variability. Within this broad range of genotypes, factors which might contribute to an increase in virulence and determine clinical outcome and severity of infection are the level of produced toxins, sporulation and germination efficiencies, as well as surface layer proteins and flagella. Furthermore, transmission of this pathogen is accomplished by the fecal-oral route by the uptake of highly resistant spores which lead to the spread and persistence of strains and could favor the prevalence of endemic ribotypes.
To get insights into the fitness, virulence and transmission of *C. difficile*, 35 clinical *C. difficile* strains from the phylogenetic clades and from different origins were tested for antibiotic susceptibility to clinical used antibiotics, *in vitro* growth, sporulation and germination capacities, as well as for toxin expression and motility. Additionally, PCR-ribotyping and total spore titers in stool samples of respective CDI patient were performed.
The results show differences in the antibiotic resistance of strains to Erythromycin and Moxifloxacin, depending on the origin. However, all tested isolates were susceptibility to Metronidazole and Vancomycin used in CDI therapy. For the growth capacities

in anaerobic batch cultures, no significant or strain-dependent differences could be observed. Accounting the CFU/ml, five strains exhibited a significant higher sporulation in comparison to reference strain 630, whereas clade 5 isolates revealed a reduced sporulation and a loss of motility. Additionally, RT 126 isolates exhibited elongated cells with defects in cell-division that is still under investigation. In conclusion, clinical isolates reveal a high diversity of adaptive mechanism, like low toxin production that could favor host survival or a higher sporulation for the host to host transmission.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

458/GIP

A nickel-regulated small RNA represses expression of multiple major virulence factors in *Helicobacter pylori*

S. Eisenbart*[1], M. Alzheimer[1], S. Pernitzsch[1], S. Stahl[1], R. Haas[2], C. Sharma[1]
[1]*University of Würzburg, Research Centre for Infectious Diseases, Würzburg, Germany*
[2]*Ludwig-Maximilians-University Munich, Max von Pettenkofer-Institute, Munich, Germany*

The Gram-negative Epsilonproteobacterium *Helicobacter pylori* colonizes the stomachs of about 50% of the worlds population and thereby leads to gastritis, ulcers, and gastric cancer. Several virulence factors including secreted effectors, exotoxins and outer membrane proteins have been described in *H. pylori* that allow this spiral-shaped bacterium to survive in the acidic environment of the human stomach and to interact with host cells. For many of these factors their contribution to pathogenicity has been well studied. However, how their expression is regulated is less understood. Whereas many genes important for pathogenicity are regulated at the transcriptional level, e.g. in response to low pH or the availability of metal ions, almost nothing is known about their regulation at the post-transcriptional level.
Based on a differential RNA-seq approach we had identified an unexpected number of more than 60 small RNA (sRNA) candidates in *H. pylori* strain 26695 (1). While their functions and targets remained largely unknown, we now report the characterization of an abundant and conserved sRNA, NikS (NikR-dependent sRNA), and show that it directly represses expression of several major virulence factors at the post-transcriptional level. Using *in vitro* and *in vivo* experiments, we demonstrate that NikS sRNA folds into a stem-loop structure with an extended loop region and directly binds to the mRNAs of multiple virulence genes with different single-stranded regions within its extended loop region. Moreover, we demonstrate that expression of NikS itself is transcriptionally repressed in response to nickel stress through the transcriptional regulator NikR. In turn, the major virulence factors are post-transcriptionally repressed through NikS sRNA dependent on nickel availability. *In vitro* cell culture infection assays revealed that deletion and overexpression of *nikS* impacts host cell interaction, indicating that fine-tuning and coordinated regulation of virulence gene expression by NikS is important for pathogenesis. Overall, NikS represents the first potential virulence regulating sRNA from *H. pylori*.

Reference

[1] Sharma, C.M., Hoffmann, S., Darfeuille, F., Reignier, J., Findeiss, S., Sittka, A., Chabas, S., Reiche, K., Hackermuller, J., Reinhardt, R. *et al.* The primary transcriptome of the major human pathogen Helicobacter pylori. *Nature*, 2010, 464, 250-255.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

459/GIP

Identification and characterization of pseudouridine in RNAs of the human pathogen *Campylobacter jejuni*

E. Fiore *[1], G. Dugar[1], H. S. Tan[1], T. Bischler[1], C. Sharma[1]
[1]*University of Würzburg, Research Center for Infectious Diseases (ZINF), Würzburg, Germany*

More than 100 types of RNA modifications have been described in all kingdoms of life. While most modifications are found in housekeeping RNAs such as rRNAs or tRNAs, recent genomics approaches have also revealed modifications in mRNAs. Here, we are investigating RNA modifications in *Campylobacter jejuni*, the currently most common cause of bacterial gastroenteritis in humans, with a focus on pseudouridine (ψ) [1,2]. ψ, the most abundant modification in tRNA/rRNA, is an universally-conserved isomer of uridine and posttranscriptionally generated by pseudouridine synthases (PUS). Recently, a novel method for global ψ profiling (so called Pseudo-seq) based on deep-sequencing of reverse transcription stops at chemically-modified ψ was developed [3-5]. This method reported ψ in human and yeast mRNAs, yet the functions of ψ are still enigmatic. Increased ψ under heat stress indicated it might modulate RNA stability/structure or even coding potential, since artificially-introduced ψ can mediate nonsense suppression (reviewed in [2]). Using unbiased genomics approaches, we aim to globally profile ψ in *C. jejuni*. Using co-immunoprecipitation combined with RNA-seq (RIP-seq) to globally study RNA substrates of the tRNA-modifying PUS enzyme TruB, we could successfully enrich for tRNAs and identify potential PUS substrates. Pseudo-seq of RNAs from *C. jejuni* wildtype (WT) and PUS mutant strains (Δ*truA*, Δ*truB,* Δ*truD*) provided a global map of ψ in tRNAs and rRNAs. Phenotypic analyses revealed an impact of Δ*truA* and Δ*truD* deletions on bacterial growth and motility. Using so-called ribosome profiling based on deep sequencing of ribosome protected fragments for global translatome analyses, we aim to investigate how deletion of PUS enzymes, and in turn depletion of ψ, affects gene expression and translation rates. Using biochemical, molecular biology, and genetics methods, we will investigate the functions of ψ in bacterial RNAs and assess changes in RNA stability, structure, and/or coding potential. The study of ψ in *C. jejuni* will provide insight into posttranscriptional regulation by RNA modifications and their function.

References

[1] Spenkuch et al. (2014) RNA Biol., 11, 1540-54.
[2] Ge & Yu (2013) Trends in Biochemical Sciences, 38, 210-218.
[3] Schwartz, et al. (2014) Cell, 159, 148-162.
[4] Carlile et al. (2014) Nature, 515, 143-146.
[5] Lovejoy et al. (2014) PLoS One, 9, e110799.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

460/GIP

CagA and VacA genotypes in peptic ulcer disease and non-ulcer dyspepsia in Iran

H. R. Baradaran*[1], H. Fakhre Yaseri[2], K. Soltani- Arabshahi[2], M. Shekaraby[3]
[1]*Iran University of Medical Sciences, Epidemiology, Tehran, Islamic Republic Of Iran*
[2]*Iran University of Medical Sciences, Research Center for Gastroenterology and Liver Disease, Department of Internal Medicine and Gastroenterology, Firoozgar Hospital,, Tehran, Islamic Republic Of Iran*
[3]*Iran University of Medical Sciences, Research Center of Immunology, Tehran, Islamic Republic Of Iran*

Question: The cag pathogenicity island includes a number of genes, including cytotoxin-associated protein A (cagA) and vacuolating cytotoxin (vacA) genotypes, which are associated with bacterial virulence. Although the role of cagA and vacA in the virulence of Helicobacter pylori (H. pylori) is well-established in epidemiological studies, the relationship between the cagA and vacA genotypes in Iran has yet to be fully elucidated. This study compared the association between cagA and vacA genotypes between peptic ulcer disease (PUD) patients and non-ulcer dyspeptic (NUD) patients.
Methods: This case control study was done on 130 patients with positive H. pylori in histological and Giemsa reports. The case group comprised 65 PUD patients, and the control group included 65 NUD patients. The presence of the cagA and vacA genotypes was determined using polymerase chain reaction (PCR) on biopsy samples, taken via endoscopy.
Results: Both cagA and vacA genotypes were positive in 51.5% (17) of the PUD group and 20% (6) of the NUD group (p= 0.009), and both cagA and vacA genotypes were negative in 48.5% (16) and 80% (24) of the case and control groups, respectively (p= 0.03). CagA-positive H. pylori was detected in 41.5% (27) and 24.6% (16) of the case and control groups, respectively (p= 0.001), and vacA-positive H. pylori was found in 60% (39) and 46% (30) of the case and control groups, respectively.
Conclusion: Both cagA and vacA genotypes were more prevalent in the PUD patients than in their NUD counterparts among our Iranian samples. It is seems that the determination of these two genotypes in PUD patients is a good screening tool for patient selection for endoscopy and treatment.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

461/GIP

Primary resistance to clarithromycin is still low in *Helicobacter pylori*

B. Bluemel*[1], H. Goelz[1], B. Eisele[1], E. Glocker[1,2]
[1]*University Hospital Freiburg, Medical Microbiology and Hygiene, Freiburg, Germany*
[2]*Brandenburg Medical School, Clinical Centre Brandenburg, Institute of Laboratory Medicine, Brandenburg/Havel, Germany*

Objectives: There are only limited data on primary antimicrobial resistance of *Helicobacter pylori* in Germany. The aim of this prospective study was to update these data and to compare the proportion of resistant strains in different populations.
Methods: From October 2014 until October 2016, a total of 1099 adult patients from Germany who had not yet received any prior eradication treatment were enrolled. Gastric tissue samples used for the Helicobacter Urease Test (HUT) were molecular genetically tested for mutations conferring resistance to clarithromycin, levofloxacin and tetracycline. Data on patients underlying gastric disease and country of origin were gathered.
Results: About 63% of the enrolled patients suffered from gastritis; 15% from peptic ulcer disease; 7% did not reveal any macroscopic alterations of the gastric mucosa. The majority of patients (53%) was born in Germany; 9.5% in Russia or other countries of the former Soviet Union; 8.5% in Turkey; 10% in Southern and/or Eastern European countries; 5% in an Asian country.
Overall, primary resistances were 10.1% for clarithromycin and 11.6% for levofloxacin; 2.2% revealed resistance and/or reduced susceptibility to tetracycline; 2.3% showed combined resistance to clarithromycin and levofloxacin. There was no significant difference in clarithromycin resistance with regards to the underlying gastric disease and patients country of origin.
Conclusions: Our data show that the prevalence of primary clarithromycin resistance is low in Germany making clarithromycin still a reasonable option for empiric eradication treatment.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

462/GIP

Large-scale screen for identification of *Campylobacter jejuni* fitness determinants promoting host colonization

B. Gao[1], H. Vorwerk[2], C. Huber[3], M. Lara-Tejero[1], J. Mohr[2], A. L. Goodman[1], W. Eisenreich[3], J. E. Galán[1], D. Hofreuter*[2]
[1]*Yale University School of Medicine, Department of Microbial Pathogenesis, New Haven, Connecticut United States*
[2]*Hannover Medical School, Institute for Medical Microbiology and Hospital Epidemiology, Hannover, Germany*
[3]*Technische Universität München, Lehrstuhl für Biochemie, Garching, Germany*

Campylobacter is a major cause of bacterial food-borne illness in humans worldwide. *Campylobacteriosis* is largely triggered by the ingestion of *Campylobacter jejuni* that persist as commensal intestinal bacteria in a broad range of livestock including chicken. Currently, effective measurements that reduce the load of *C. jejuni* in its primary hosts are missing, and attempts to develop universal vaccines against surface-exposed structures have not been successful due to the strain-dependent variability in traditional targets like capsule or lipooligosaccarides.
Additional methods are required to fight *C. jejuni* infection, and approaches that lower the quantitative burden of *C. jejuni* in the food supply and consequently decrease human infections are promising approaches to combat this prevalent pathogen. Our current knowledge about factors that allow *C. jejuni* to proliferate efficiently in its hosts is limited. Therefore, we used a genome-wide approach to explore the properties of *C. jejuni* that facilitate its intestinal colonization. Combining non-targeted mutagenesis and metabolomic techniques, we discovered and characterized physiological traits that are required by a highly pathogenic *C. jejuni* strain for the colonization of a murine model of infection. This comprehensive investigation has identified new targets that could provide the basis for the development of novel strategies to combat *Campylobacteriosis* by reducing *C. jejuni* colonization in food-producing animals.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

463/GIP

The anti-inflammatory drug mesalamine targets bacterial polyphosphate accumulation

J. U. Dahl*[1], M. J. Gray[1], U. Jakob[1]
[1]*University of Michigan, Department of Molecular, Cellular and Developmental Biology, Ann Arbor, Michigan, United States*

Polyphosphate (polyP) is an ancient, universally conserved biopolymer, composed of linear chains of up to 1000 inorganic phosphate monomers. While polyP is found in cells of all three domains of life, only in bacteria have the enzymes of polyP metabolism been well studied. Microbial polyP synthesis is catalyzed by polyP kinase (PPK), an enzyme almost exclusively

found in bacteria. Bacteria lacking PPK are defective in virulence, biofilm formation, persistence, and oxidative stress response, making PPK a potentially powerful antimicrobial drug target. We therefore developed an *in vitro* PPK assay and screened small molecule libraries for PPK inhibitors. One of our identified PPK-inhibitors turned out to be 5-aminosalicylic acid (a.k.a. mesalamine), a drug that has been used to treat patients with mild to moderate ulcerative colitis (UC) for over 70 years. UC is characterized by chronic inflammation of the colon resulting in changes in its microbiome. Mesalamine, which works on the luminal side of the inflamed colonic mucosa, effectively decreases inflammation. While its mechanisms of action(s) are still largely unknown, recent work suggested that in addition to affecting host responses, mesalamine might also alter adhesion and persistence of intestinal gut bacteria, leading to a reduced load of mucosal bacteria in mesalamine-treated UC patients. We therefore hypothesized that mesalamine might contribute to this effect by modulating the polyP content of pathogenic bacteria and potentially sensitize them to stress, including antibiotics, disinfectants, and attack by the immune system.
Kinetic studies using purified *E. coli* PPK confirmed that mesalamine inhibits PPK *in vitro* by increasing PPKs KM for ATP and lowering its Ki for substrate inhibition by ATP. Treatment with a non-lethal dose of mesalamine resulted in a 50-60% reduction in polyP levels in a variety of PPK-containing pathogens and bacteria cultivated from the cecal content of healthy mice suggesting that mesalamine directly affects bacterial polyP levels *in vivo*. We found that mesalamine treatment i) caused a dose-responsive increase in HOCl sensitivity of uropathogenic *E. coli*; ii) quite faithfully reproduced in diverse wild-type strains the defect in biofilm formation; iii) reduced formation of ampicillin-resistant persister cells; and iv) decreased the ability of wild-type strains to colonize *Caenorhabditis elegans,* notably, in all cases to a level comparable to the phenotype observed in the respective *ppk*-deficient strain**.** In neither case did mesalamine treatment affect the phenotype of the respective *ppk* deletion strain, indicating that the observed effects are indeed PPK dependent. To determine the levels of mesalamine that gut bacteria encounter during treatment phases and how they affect microbial polyP levels *in situ*, we treated healthy human subjects with clinically relevant doses of two commonly used mesalamine-based drugs. Consistent with our data, the moment measurable amounts of mesalamine were detected in the samples, the polyP levels were dramatically decreased. Bacteria cultivated from human fecal content, a commonly used substitute for colon microbiome samples, were equally responsive to mesalamine treatment. These results strongly suggest that microbiota-encoded PPK is indeed a physiologically relevant target of mesalamine in humans.
Our study demonstrated that mesalamine affects polyP levels in a wide variety of different bacteria ranging from clinically isolated UPEC and *P. aeruginosa* strains to gastrointestinal luminal samples. These results suggest that mesalamines effects in treating UC is not only due to its anti-inflammatory action but also by directly altering the ability of bacteria to colonize, survive and persist within an environment of chronic inflammation.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

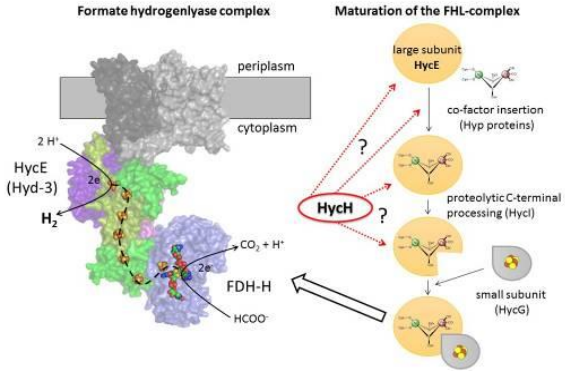
POSTERSESSION
General Metabolism (FG GMB)

464/GMBP
Spatiotemporal assembly and function of the modular formate hydrogenlyase complex
P. Skorupa*[1], U. Lindenstrauß[1], C. Pinske[1]
[1]*Martin-Luther-Universität Halle-Wittenberg, Institute of Biology/ Microbiology, Halle (Saale), Germany*

Escherichia coli is a facultative anaerobic bacterium whose genome encodes four hydrogenases, three of which have been functionally characterised. *E. coli* is able to produce hydrogen through the oxidation of formate when grown on glucose and in the absence of external electron acceptors. This reaction is catalysed by the seven-subunit, membrane-associated formate hydrogenlyase (FHL) complex[1]. One of the two catalytic subunits within the cytoplasmic domain is formate dehydrogenase H (formate DH). The protein harbours a molybdenum cofactor where formate is oxidised to CO_2 and electrons. The second catalytic subunit is a [NiFe]-hydrogenase that uses these electrons to reduce protons, thus generating hydrogen. Previous work has established that a pool of accessory proteins (called Hyp proteins) are needed for the synthesis and insertion of the $NiFe(CN)_2CO$ cofactor during maturation of the FHL complex.
The electrons are transferred between both catalytic subunits by three iron-sulfur proteins. The cytoplasmic domain of the FHL complex is attached to the inside of the membrane by two further integral membrane subunits.
Harnessing hydrogen generated by the FHL-complex is a promising means for biotechnological H_2 production. For this reason we wish to understand how the complex is assembles and its mechanism.
We employ microbiological methods to generate a variety of mutants and monitor their effect on hydrogen production. Furthermore, we analyse the FHL-complex with biochemical methods in order to identify interaction partners. For example, how the formate-DH interacts with the hydrogenase and the iron-sulfur proteins remains to be characterized.
It is possible to purify the complete complex or sub-complexes thereof[2,3], but the order of events during assembly is not yet understood. For example, the function of the additional accessory protein HycH remains unknown. We used analytical SEC in combination with SDS-PAGE to analyse the sub-complexes. HycH interacts with the large hydrogenase subunit HycE during assembly of the complex, but it is not a structural subunit of the final FHL complex. Studies of the interaction show that an exchange of conserved tyrosine residues can interrupt the interaction, while exchange for histidines abolishes activity. In addition, deletion of the *hycH* gene results in a reduction of FHL activity to 30 %. Expression and co-purification of HycE/HycH in different backgrounds allow monitoring of the interaction.
HycH might play an important role in regulation of FHL complex assembly and its activity is still unclear. Understanding FHL assembly will be great benefit for the biotechnological exploitation of its hydrogen-producing capability.

References
[1] Sauter, M., Böhm, R. & Böck, A. *Mol Microbiol* 6, 1523–1532 (1992)
[2] McDowall, J. S., Hjersing, M. C., Palmer, T. & Sargent, F. *FEBS Lett* 589, 3141–3147 (2015)
[3] McDowall, J. S. *et al. Proc Natl Acad Sci USA* 111, E3948–56 (2014)

Figure 1



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

465/GMBP

TsdC, a unique lipoprotein from *Wolinella succinogenes* that enhances tetrathionate reductase activity of TsdA

J. Kurth*[1], A. Schuster[1], W. Seel[1], S. Herresthal[1], J. Simon[2], C. Dahl[1], S. Herresthal[1]
[1]*Rheinische Friedrich-Wilhelms-Universität Bonn, Institut für Mikrobiologie & Biotechnologie, Bonn, Germany*
[2]*Technische Universität Darmstadt, Microbial Energy Conversion and Biotechnology, Darmstadt, Germany*

The diheme cytochromes *c* of the widespread TsdA family are bifunctional thiosulfate dehydrogenase/tetrathionate reductases [1, 2, 3, 4]. Organisms like the purple sulfur bacterium *Allochromatium vinosum* use TsdA to oxidize two molecules of thiosulfate to yield tetrathionate [1, 2]. On the other hand, TsdA enables prokaryotes like the human gut pathogen *Campylobacter jejuni* to use tetrathionate as alternative electron acceptor [3]. Here, biochemical information was collected about TsdA from the Epsilonproteobacterium *Wolinella succinogenes* (*Ws*TsdA). The situation in *W. succinogenes* is unique since TsdA is closely associated with the unprecedented lipoprotein TsdC encoded immediately downstream of *tsdA* in the same direction of transcription. TsdC does not show any significant sequence similarity to proteins from other organisms. The identity of TsdC as a lipoprotein is indicated by its membrane localization in *W. succinogenes* as well as in *Escherichia coli* and the presence of a signal peptide typical for lipoproteins. This is supported by the finding that the N-terminus is posttranslationally modified as suggested by our failure to detect any unmodified amino-terminal peptides in a mass spectrometric approach and by the observation that the N-terminus of TsdC isolated from *W. succinogenes* was blocked upon Edman degradation. Motifs for binding of prosthetic groups that might mediate electron transfer are not apparent in this protein. TsdC was found to form a tight complex with TsdA when purified from *W. succinogenes* as well as from *E. coli* membranes. *Ws*TsdA purified from *E. coli* catalyzed both thiosulfate oxidation and tetrathionate reduction, but showed very low specific activities. After co-production of TsdC and *Ws*TsdA in *E. coli*, TsdC was found to mediate membrane attachment of TsdA and to ensure its full catalytic activity. This effect was much stronger in the tetrathionate-reducing than in the thiosulfate-oxidizing direction. It is concluded that the TsdAC complex predominantly acts as a tetrathionate reductase *in vivo*.

References

[1] Denkmann *et al*. (2012) Environ. Microbiol. 14, 2673-2688
[2] Brito *et al*. (2015) J. Biol. Chem. 290, 9222-9238
[3] Liu *et al.* (2013) Mol. Microbiol. 88, 173-188
[4] Kurth *et al*. (2015) J. Am. Chem. Soc. 137, 13232-13235

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

466/GMBP

Isolation of Chlorate-Resistance Mutants Identifies New genes involved in *Streptomyces* Nitrate Reduction

M. Weidt*[1], C. Naujoks[1], E. Zahn[1], G. Sawers[1], M. Fischer[1]
[1]*Martin Luther Universität, Halle, Germany*

Introduction: Streptomycetes are soil-dwelling bacteria that require oxygen for growth. Nevertheless, under hypoxic conditions various representatives of this genus perform respiratory nitrate reduction to insure maintainance of their membrane potential. *S. coelicolor* A3(2) synthesizes three respiratory nitrate reductases (Nars). Nar2 is the main enzyme active in exponentially growing mycelium. Synthesis of Nar2 is regulated by hypoxia but not by nitrate. Only one regulator for the synthesis of Nar2, OsdR, is currently known and recent evidence suggests it might sense hypoxia. The activity of all Nar and other molybdo-enzymes depends on a bis-molybdopterin guanine dinucleotide (bisMGD) -cofactor. A classical strategy to isolate mutants defective in nitrate reduction or bisMGD synthesis involves selection for growth in the presence of the nitrate analogue chlorate, which when reduced produces toxic chlorite. Isolation of chlorate-resistant mutants presents a useful strategy to identify new genes involved in nitrate reduction.
Objectives: Because regulation of Nar2 synthesis is poorly understood we wish to isolate new mutants with defects specifically in Nar2 synthesis and activity. To do this we wished to optimize our selection strategy and make use of a random transposon library of *S. coelicolor* to isolate new chlR-resistant mutants.
Materials & methods: We screened a transposon library of *S. coelicolor* spores for chlorate- resistant germinating cells*. The selected mutants were initially characterized for their ability to grow on nitrate as sole nitrogen source and to reduce chlorate and nitrate *in vivo*. Afterwards crude extracts of cells were tested for bisMGD-cofactor- dependent enzyme activities like xanthine oxidases, aldehyde dehydrogenases and nitrate reductases.
Results: As anticipated, we identified random transposon insertions in *nar*-operons as well as in genes of the bisMGD-cofactor biosynthetic pathway, like *moaC*, which demonstrates that our selection strategy worked. As well as these mutants, we identified a class of mutants unable to reduce chlorate but which were still capable of nitrate reduction. Another class of mutants were defective specifically in Nar2 synthesis. Finally, 4 other mutant classes were identified with so far uncharacterized genes required for Nar2 activity.
Conclusion: We have identified several previously uncharacterized genes whose products are involved in the regulation, synthesis or activity of the Nar2 enzyme of *S. coelicolor* A3(2). Characterization of these genes will provide new insights into the regulation of Nars in the obligately aerobic streptomycetes,, which differs fundamentally from the well-known regulation in facultatively anaerobic bacteria.
*provided by Keith Chater

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

467/GMBP

Proteome-based new insights into dissimilatory sulfur metabolism of *Hyphomicrobium denitrificans*

T. Koch*[1], C. Dahl[1]
[1]*Rheinische Friedrich-Wilhelms-Universität , Institut für Mikrobiologie & Biotechnologie , AG Dahl, Bonn, Germany*

Bacteria of the genus *Hyphomicrobium* are well known for their capacity to grow on C1 compounds like methanol or methylamine. Furthermore, some species can grow on volatile sulfur compounds and/or gain additional energy from the oxidation of inorganic sulfur compounds like thiosulfate (1). Previously, we reported the ability of *Hyphomicrobium denitrificans* (DSM 1869^T) to grow on dimethylsulfide (DMS) as sole carbon and energy source associated with full oxidation of the sulfur contained in DMS to sulfate. Here, we present first proteome-based insights into the pathway of DMS oxidation with a focus on the fate of the sulfur atom.

To this end, the proteome of *Hyphomicrobium* cells grown on DMS or dimethylamine (DMA) was compared via Liquid Chromatography-Mass Spectrometry. While grown on DMS, proteins for assimilatory sulfur metabolism were much less abundant than in cells using DMA as carbon and sulfate as sulfur source. Simultaneously, enzymes catalyzing crucial steps in the initial degradation of DMS like a candidate for a cytoplasmic DMS monooxygenase and the periplasmic methanethiol oxidase that releases sulfide and formaldehyde as products (2) were identified and found to be much more abundant in DMS- than in DMA-grown cells. In addition to sulfide:quinone oxidoreductase and persulfide dioxygenase, proteins SoxB, SoxXA and SoxYZ constituting parts of the periplasmic thiosulfate-oxidizing Sox multienzyme complex were highly abundant on DMS, suggesting thiosulfate as a potential intermediate during aerobic DMS oxidation. Since the genome of *H. denitrificans* also harbors a copy of SoxCD, a complete oxidation of thiosulfate to sulfate via the Sox system would in principle be possible. However, SoxCD was not detected under any of the tested growth conditions. Most notably, proteins encoded in the heterodisulfide reductase (*hdr*)-like gene cluster, which are suspected to play a crucial role in cytoplasmic oxidation of sulfane sulfur in prokaryotes lacking the well-established Dsr pathway (3), were up to 40-fold more abundant in DMS than in DMA grown cells. The *hdr*-like genes are not only linked with genes encoding sulfur-trafficking proteins like TusA but also with a gene for a lipoate-binding protein (2.5- and 7-fold up on DMS), supporting a prominent role of lipoate within the Hdr-like system of sulfur oxidation (4). Taken together our findings strengthen the notion that a Hdr-like complex is involved in a process functionally replacing the Dsr system and indicate a potential interplay between the Hdr-like and the Sox system during the oxidation of DMS-derived sulfur.

References

[1] Suylen GMH, Stefess GC, Kuenen JG. (1986) *Arch. Microbiol.* 146, 192
[2] Suylen GMH, Large PJ, van Dijken JP, Kuenen JG. (1987) *Microbiol.* 133, 2989
[3] Venceslau SS, Stockdreher Y, Dahl C, Pereira IA. (2014) *BBA* 1837, 1148
[4] Liu LJ, Stockdreher Y, Koch T, *et al*. (2014) *J. Biol*. Chem. 289, 26949

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

468/GMBP

A Hydrogen-Oxidizing, Organohalide-Reducing Respiratory Complex in *Dehalococcoides mccartyi*

N. Krumova*[1], S. Hartwig[1], K. Nutschan[1], G. Sawers[1]
[1]*Martin Luther University Halle-Wittenberg, Microbiology, Halle (Saale), Germany*

Introduction: Strains of the deeply-branching, strictly anaerobic bacterium *Dehalococcoides mccartyi* are characterized by their dependence on organohalide respiration (OHR) for growth. Hydrogen is so far the only known electron donor for OHR in this species. The bacterium is also unusual in that it does not have quinones, and recent evidence suggests that a respiratory super-complex is responsible for H2-driven OHR (Kublik et al., 2016).
Despite its compact genome (*ca*. 1.4 Mbp) the bacterium has the coding capacity for several different classes of NiFe-hydrogenases, as well as up to 36 different reductive dehalogenases (Rdh), depending on the strain. Along with these hydrogenases and Rdhs, the bacterium also encodes a large and highly abundant predicted molybdoenzyme, which has been termed CISM (complex iron-sulfur molybdoenzyme; Kublik et al., 2016). The function of CISM is unknown but it forms a complex with an uptake hydrogenase (Hup) as well as different Rdhs, which suggests, it might have a function related to OHR. Hup, Rdh and CISM are all Tat substrates and the catalytic site of all three enzymes is located on the outer leaflet of the cytoplasmic membrane.
Objectives: First, we wish to characterize OHR catalyzed by the Hup-CISM-Rdh supercomplex. Second, using heterologous expression we aim to determine whether each of these normally membrane-associated proteins can be inserted into the membrane of *E. coli*.
Methods: A mutant unable to synthesize all three characterized NiFe-hydrogenases of *E. coli* was used for heterologous studies. Peptide-specific antibodies were used to analyze protein levels in *E. coli*. Protein complexes in crude extracts derived from *D. mccartyi* grown under OHR conditions using 1,2,3-trichlorobenzene (1,2,3-TCB) were separated by various chromatographic methods and analyzed for the ability to dechlorinate 1,2,3-TCB.
Results: OHR and Hup enzyme activities could be determined after separation of protein complexes by gel-filtration and anion-exchange chromatography. Mass spectrometric analysis revealed that some of these complexes included Hup, CISM and Rdh. Heterologous expression analyses conducted in *E. coli* revealed that Hup hydrogenase is active and maturation of the enzyme relied on the host"s maturation system. Moreover, the active hydrogenase showed Tat-dependent membrane association. Despite not knowing the biochemical function of the CISM enzyme, this enzyme could also be successfully transported across the membrane by the Tat-system in *E. coli*.
Conclusion: Two components of a putative OHR protein complex enriched from crude extracts of *D. mccartyi* have been successfully synthesized and transported via Tat machinery into the membrane of *E. coli*. This latter result indicates that CISM and Hup were correctly folded and is the first step towards functional reconstitution of OHR in *E. coli*.

References

[1] Kublik et al. (2016) *Environ. Microbiol.* 18: 3044-3056.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

469/GMBP

New insights into cAMP dependent regulation in *Corynebacterium glutamicum*

J. Schulte[1], N. Wolf*[1], M. Baumgart[1], M. Bott[1]
[1]*Forschungszentrum Jülich, IBG-1, Jülich, Germany*

Question: The transcriptional regulator GlxR plays a central role in the regulation of metabolism in the industrial workhorse *Corynebacterium glutamicum* because it activates or represses more than 14 % of all genes [1]. To bind DNA, GlxR has to form a complex with the second messenger cyclic adenosine monophosphate (cAMP). Therefore, knowledge how cells control the cellular cAMP level by cAMP-synthesizing and -degrading enzymes is the key to gain physiological understanding of transcriptional regulation by GlxR. In *C. glutamicum* so far only the cAMP-synthesizing enzyme adenylate cyclase CyaB has been described [2]. This project focused on the investigation of the cAMP-degrading phosphodiesterase CpdA [3].
Methods: CpdA was identified as a phosphodiesterase (PDE) and the purified enzyme was characterized with different enzymatic assays. A *cdpA* deletion mutant was generated and growth experiments in the Biolector microcultivation system were performed. Intracellular cAMP concentrations were measured with a cAMP-specific ELISA. Global gene expression was modified by DNA microarray analysis. Promoter fusions with the *venus* reporter gene encoding a fluorescent protein were used for analyzing transcriptional regulation of *cpdA*.
Results: The cAMP-PDE activity of CdpA was demonstrated *in vivo* and *in vitro*. The purified enzyme has a value of 2.5 ± 0.3 mM for cAMP and a of 33.6 ± 4.3 µmol min-1 mg-1. The Δ*cpdA* mutant at least twofold altered mRNA levels for 82 known GlxR target genes. The Δ*cpdA* mutant showed growth defects on all tested carbon sources (glucose, acetate, gluconate, citrate), which could partially or completely be abolished by plasmid-based overexpression of genes for transporters or catabolic enzymes that showed decreased mRNA levels in the mutant. Expression of the *cpdA* gene was found to be activated by GlxR, providing a feedback-loop that counteracts increased cAMP levels.
Conclusion: CpdA was characterized as cAMP-phosphodiesterase and shown to be a key enzyme in the control of the intracellular cAMP level in *C. glutamicum*.

References

[1] Kohl & Tauch (2009) J. Biotechnol. 143: 239-246
[2] Cha et al. (2010) Appl. Microbiol. Biotechnol. 85: 1061-1068
[3] Schulte et al. (2016) DOI: 10.1111/mmi.13574

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

470/GMBP

Electron transfer from bifurcating Etf to butyryl-CoA dehydrogenase in the complex from *Clostridium difficile*

J. K. Demmer[1], N. P. Chowdhury[2], U. Ermler[1], T. Selmer[3], W. Buckel*[2]
[1]*Max-Planck-Institute für Biophysik, Frankfurt am Main, Germany*
[2]*Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany*
[3]*Technical University, Biology, Aachen, Germany*

Flavin-based electron bifurcation (FBEB) is a recently recognized process, which enables strict anaerobic bacteria and archaea to reduce ferredoxin or flavodoxin by using donors with higher redox potentials (1). In the clostridial system composed of heterodimeric electron transferring flavoprotein (EtfAB) and tetrameric butyryl-CoA dehydrogenase (Bcd) the two electrons of NADH bifurcate. One electron goes to the high potential crotonyl-CoA and the remaining highly reactive electron reduces the low potential ferredoxin or flavodoxin. Repetition of this process affords butyryl-CoA and a second reduced ferredoxin or flavodoxin (2). These "energy rich" electron carriers either form up to 100 kPa hydrogen or generate an electrochemical sodium ion gradient for ATP synthesis mediated by ferredoxin-NAD reductase, also called Rnf (3).
Characterization of EtfAB from *Acidaminococcus fermentans* revealed a three domain structure containing two FAD. α-FAD is located on the flexible domain II and β-FAD sits between domain I and III, close to the NADH binding site. Though the distance between the FADs amounts 18 Å, movement of domain II without disturbing the tertiary structure shortens the distance to 14 Å allowing a fast electron transfer. The binding site of ferredoxin was modeled close to β-FAD, only 6.5 Å apart. It is proposed that in this conformation NADH transfers a hydride to the bifurcating β-FAD of Etf, which donates one electron to α-FAD of Etf and the other to ferredoxin. The formed α-FAD•- semiquinone hands the electron over to d-FAD of Bcd, where crotonyl-CoA is reduced to butyryl-CoA (3).
The structure of the heterododecameric (EtfAB-Bcd)4 complex from *Clostridium difficile* revealed a tetrameric Bcd core surrounded by four Etfs. Thereby domain III of each Etf binds to one Bcd subunit and domain II has rotated by 78° in such a way that the α-FAD interacts with d-FAD of the adjacent Bcd subunit, ready to transfer the electron. Hence domain II of Etf in complex with Bcd exists in two states. In the B-state, α-FAD receives the electron by bifurcation from β-FAD and in the D-state α-FAD•- donates the electron to d-FAD. As shown in the structure, the D-state is also the resting state of the Etf-Bcd complex. Either domain II randomly swings back and forth to the B-state like a seesaw, or binding of NADH induces a movement of the domain II-embracing C-terminal arm of EtfB, which restores the B-state to start the next round of bifurcation.

References

[1] Buckel, W., and Thauer, R. K. (2013) *Biochim. Biophys. Acta* 1827, 94-113.
[2] Chowdhury, N. P., Mowafy, A. M., Demmer, J. K., Upadhyay, V., Koelzer, S., Jayamani, E., Kahnt, J., Hornung, M., Demmer, U., Ermler, U., and Buckel, W. (2014) *J. Biol. Chem.* 289, 5145-5157.
[3] Chowdhury, N. P., Klomann, K., Seubert, A., and Buckel, W. (2016) *J Biol Chem* 291, 11993-12002.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

471/GMBP

RNA-sequencing reveals selective adaptation of *Streptococcus suis* to porcine blood and cerebrospinal fluid

A. Koczula*[1], M. Jarek[2], C. Visscher[3], P. Valentin-Weigand[1], R. Goethe[1], J. Willenborg[1]
[1]*University of Veterinary Medicine, Institute for Microbiology, Hannover, Germany*
[2]*Helmholtz Centre for Infection Research, Genome Analytics, Braunschweig, Germany*
[3]*University of Veterinary Medicine, Institute of Animal Nutrition, Hannover, Germany*

Introduction: *Streptococcus* (*S.*) *suis* is a zoonotic pathogen that can cause severe pathologies such as septicemia and meningitis in pigs as well as in humans. It is suggested that the first step for infection is the colonization of the upper respiratory tract in pigs. These colonizing bacteria may enter the bloodstream and further the cerebrospinal fluid (CSF) which might be associated with central nervous infection. The establishment of invasive disease depends not only on the expression of virulence factors but also on metabolic genes due to the adaptation to different nutrients in these specific host environments. How the streptococcal metabolism adapts to the host milieu encountered is poorly understood.
Objectives: Our previous isotopologue profiling studies on *S. suis* grown in porcine blood and CSF revealed conserved activities of central carbon metabolism in both body fluids but they suggested

differences in the *de novo* amino acid biosynthesis. In order to investigate metabolic differences in more detail, the transcriptomic adaptation of *S. suis* to porcine body fluids such as blood and CSF was analyzed on a gene expression level.
Methods: RNA deep sequencing was conducted of bacteria grown in porcine blood, porcine CSF and standard laboratory medium to detect genes important for *ex vivo* adaptation of *S. suis* to dissect preferred metabolic pathways. RNA-sequencing data were additionally validated by RT-qPCR.
Results: In blood, the most differentially expressed genes were associated with carbohydrate metabolism (pentose phosphate pathway, glycogen metabolism) and transport of alternative carbohydrate sources. Remarkably, differential expression of several genes could be linked to the activity of the catabolite control protein A (CcpA) indicating that this regulator protein is involved in the adaptation process to blood.
In CSF, the majority of differentially expressed genes are related to the amino acid metabolism and transport, especially genes involved in the biosynthesis of branched chain and aromatic amino acids. Isoleucine biosynthesis seems to be of major importance for *S. suis* in CSF because several related biosynthetic genes were higher expressed.
Conclusion: In conclusion, our data revealed that adaptation of *S. suis* to host environments containing different nutrients includes prominent changes in expression of metabolic genes suggesting their importance for establishment of an infection.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

472/GMBP
New insights into the energy conserving system of *Methanomassiliicoccus luminyensis* prove the possibility of ferredoxin oxidation by the "headless" Fpo complex
L. Kröninger*[1], A. C. Böhringer[1], U. Deppenmeier[1]
[1]*University of Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*

Little is known about the central metabolism and the mode of energy conservation in *Methanomassiliicoccus luminyensis*, a member of the recently discovered order of the Methanomassiliicoccales. The organism strongly depends on H_2 as electron donor and forms methane from methylated coumpounds such as methanol or methylamines.
Based on genome analyses several enzymes were predicted to be involved in the energy conserving system of *Mmc. luminyensis* (Lang *et al.* 2015). However, deeper insights into the metabolism of *Mmc. luminyensis* were largely missing. Therefore, we focussed on the examination of methanogenic key enzymes on the enzymatic level and used a broad range of different techniques to analyze these enzymes in detail.
To get a first overview about the expression levels of genes encoding methanogenic key enzymes RT-qPCR was performed (Kröninger *et al.* 2016). Furthermore, the absence of active membrane-bound hydrogenases (Ech-type) was verified by *in gel*-hydrogenase assays and by photometric analyses. In contrast, the activity of the soluble hydrogenase MvhADG could clearly be shown. This hydrogenase is a main player of the central redox metabolism being responsible for H_2 oxidation. Additionally, disulfide reductases (HdrABC and HdrD) play a crucial role in the metabolism of *Mmc. luminyensis* as revealed by RT-qPCR. Catalytically active HdrD was overproduced in *E. coli* and the enzyme was purified and characterized. The activity of the second heterodisulfide reductase HdrABC was measured in the cytoplasmic fraction of *Mmc. luminyensis*. However, the most interesting methanogenic enzyme is the Fpo complex. While the complex oxidizes $F_{420}H_2$ in other methanogenic Archaea, it lacks the $F_{420}H_2$-oxidizing subunit in *Mmc. luminyensis*. We could show for the first time that the "headless" Fpo complex oxidizes reduced ferredoxin and transfers electrons to HdrD. The reaction is probably coupled to the generation of an electrochemical ion gradient. Experiments with resting cells and the protonophor SF6847 as well as the sodium ionophor ETH157 indicated that this ion gradient is established by protons only. This feature is unique because all known methanogenic Archaea possess a methyl transferase (Mtr) that translocates sodium ions across the membrane. Interestingly, this enzyme is missing in *Mmc. luminyensis*.
Although methanogenic metabolism has been studied extensively in the past decades the mode of energy conservation in *Mmc. luminyensis* still offers great opportunities for research. Especially the ferredoxin oxidation step at the Fpo complex seems to be widespread in nature as there is a vast number of Archaea and Bacteria possessing "headless" complex I derivatives.

References
[1] Kröninger *et al.* (2016) FEBS J. 283: 472-483.
[2] Lang *et al.* (2015) Appl. Environ. Micbrobiol. 81: 1338-1352.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

473/GMBP
A Sulfur Oxygenase from the Haloalkaliphilic Bacterium *Thioalkalivibrio paradoxus* with atypically low Reductase Activity
P. Rühl*[1], U. Pöll[1], J. Braun[1], A. Klingl[1], A. Kletzin[1]
[1]*Technische Universität Darmstadt, Mikrobiologie und Archaea, Darmstadt, Germany*

Introduction: Sulfur oxygenase reductases (SOR) catalyze a dioxygen-dependent disproportionation of elemental sulfur with sulfite, thiosulfate and sulfide as products. Usually, oxygenase and reductase reactions cannot be separated with molar ratios between 4:1 and 10:1. SORs were initially found in thermoacidophilic sulfur-oxidizing Archaea like *Acidianus ambivalens* (*Aa*SOR). It was found later that SORs characterized from mesophilic and thermophilic bacteria had comparable properties to the archaeal ones (T_{Opt}: 65-85 °C; pH_{Opt}: 5-8.4). Recently, *sor* genes were found in three haloalkaliphilic *Thioalkalivibrio* species isolated from soda lakes. Among these, *T. paradoxus* Arh1 encodes a SOR (*Tp*SOR) that branches deeply in a phylogenetic dendrogram of this protein family with only 29 % sequence identity with homologs from other microorganisms.
Objectives: This study describes properties of the *Tp*SOR as a first example of such an enzyme from a mesophilic and (halo-) alkaliphilic bacterium (growth at 30-37 ˚C and pH 10), particularly with regard to the question whether the deeply branching protein has SOR activity and whether the subgroups observed in the dendrogram represent the evolution of paralogs.
Material & Methods: The *T. paradoxus sor* gene was heterologously expressed in *E. coli*. Specific enzyme activities of the protein were determined by colorimetric assays and HPLC. Protein stability and thermal unfolding were recorded by circular dichroism spectroscopy, differential scanning fluorimetry and activity assays. The molecular mass of the enzyme was determined by gel filtration, its diameter was measured in electron micrographs.
Results: Electron micrographs and gel filtration suggest that the *Tp*SOR forms large hollow ball-shaped homo-oligomeric structures of 15 nm in diameter similar to other SORs. Sulfite and thiosulfate were formed in a temperature range of 10-98 °C (T_{Opt}: 80 °C), a pH range of 6-11.5 (pH_{Opt}: 9) and a maximum specific activity of ≈350 U/mg of protein. In contrast, the sulfide-producing reductase activity had 0.3 % of the oxygenase activity resulting in a molar ratio of ≈300:1. The melting point of the *Tp*SOR was 80 °C, however denaturation kinetics were slow: 55 % of the residual activity was retained after 25 min of incubation

at 80 °C. Site-directed mutagenesis of active site residues showed that Cys_{44} is indispensable, whereat alanine substitution of two other conserved cysteines resulted in 0.5 % residual activity.
Conclusion: The *Tp*SOR characterized here has similar biochemical and structural properties compared to other SORs. The low reductase activity points to a slightly different reaction mechanism. Hence, the *Tp*SOR represents a sulfur oxygenase, in which the reductase activity represents a mere side effect of the main oxygenase reaction and not an integral part of the reaction mechanism.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

474/GMBP
Insights into the central energy metabolism of the important human gut microbe *Prevotella copri*
T. Franke*[1], A. Augsburg[1], L. Garschagen[1], U. Deppenmeier[1]
[1]*University of Bonn, Institute for Microbiology and Biotechnology, Bonn, Germany*

The human gut contains a vast amount of microbial species which interact comprehensively with their host´s metabolism thus contributing significantly to both human health and disease. While the composition and diversity of the gut microbiome changes over time due to different diets and drug intake, it is, however, possible to distinguish between three distinct enterotypes that represent specific states of the microbial community. These enterotypes are dominated by either *Prevotella spp.*, *Bacteroides spp.* or *Ruminococcus spp.* (Arumugam *et al*., 2011). Although current research already associates these enterotypes with different medical conditions, little is known about the physiology of the stated main players of the human gut. Interestingly, there are only two *Prevotella* species known to inhabit the human colon from which *Prevotella copri* is the most abundant one (Hayashi *et al*., 2007). In fact, the Human Microbiome Project has revealed samples of individuals which contained up to 80 % *P. copri* thereby displaying an intestinal fermentation almost fully executed by a single organism. Furthermore, these samples showed a significant reduction in *Bacteroides spp.* as well as in other genera generally described as beneficial for their host. The consistently mentioned correlation between *P. copri* and rheumatoid arthritis additionally makes the organism an attractive subject for studying microbial dysbiosis and physiological adaptations towards a surprisingly unstable environment.
Here we shed light on the central energy metabolism of *P. copri* using bioinformatical, biochemical and cultural approaches to give first insights into the basic metabolic features of this relevant gut microbe. *P. copri* converts glucose via Embden-Meyerhof-Parnas pathway possessing two phosphofructokinases which are ATP or PPi dependent, respectively. Phosphoenolpyruvate is further converted to fumarate which acts as the terminal electron acceptor in the respiratory chain leaving succinate as the major fermentative end product. Electrons are channeled into the respiratory chain by a NADH:quinole oxidoreductase (Nqr) or a "headless" complex I (NDHI) which lacks the NADH converting module and potentially uses reduced ferredoxin instead of NADH. A ferredoxin:NAD^+ oxidoreductase (Rnf) and a cytochrome bd quinol oxidase are also encoded in the genome but could not be detected under the examined conditions. Further fermentative products derived from pyruvate are acetate and formate that are produced by a pyruvate:ferredoxin oxidoreductase or a pyruvate:formate lyase, respectively.

References
[1] Arumugam *et al*. (2011). Nature 473, 174-180.
[2] Hayashi *et al*. (2007). Int J Syst Evol Microbiol 57, 941-946.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

475/GMBP
New insights into the essential role of DsrL during sulfur oxidation in *Allochromatium vinosum*
M. I. Löffler*[1], J. Feldhues[1], S. S. Venceslau[2], A. A. Santos[2], I. A. C. Pereira[2], C. Dahl[1]
[1]*Universtität Bonn, Institut für Mikrobiologie und Biotechnologie , Bonn, Germany*
[2]*Universidade Nova de Lisboa, Instituto de Tecnologia Química e Biológica António Xavier, Oeiras, Portugal*

Currently, the best studied cytoplasmic sulfur oxidation pathway in dissimilatory sulfur-oxidizing prokaryotes is the so-called Dsr pathway involving the enzyme reverse dissimilatory sulfite reductase (DsrAB). It is typically preceded by the accumulation of zero-valent sulfur as a transient product during the oxidation of sulfide, thiosulfate, or polysulfides. In the purple sulfur bacterium *Allochromatium vinosum* an extensive sulfur relay system traffics sulfur atoms stemming ultimately from stored sulfur onto the protein DsrC [1]. Recent insights into the Dsr pathway in sulfate reducers [2] lead us to hypothesize that the sulfur is presented to DsrAB as a DsrC trisulfide, i.e. enclosed between the sulfur atoms of two conserved cysteines. DsrAB would then catalyze formation of sulfite and release of reduced DsrC, yielding two electrons. The fate of these electrons is completely unclear. On the other hand, it is well documented that the protein DsrL is absolutely essential for sulfur oxidation in *A. vinosum* but its function could not yet be assigned [3].
Here we show that DsrAB and DsrL are co-purifed from *A. vinosum* using classical chromatographic methods, indicating strong and specific interaction of the proteins. The N-terminal [FeS] and the central FAD-binding domains of DsrL similar to NfnB, a subunit of the NADH-dependent reduced ferredoxin:NADP oxidoreductase (NfnAB) from *Thermotoga maritima*, an established FAD-coupled electron bifurcating enzyme [4]. With the exception of one cysteine all residues coordinating the two [4Fe4S] clusters in the N-terminal domain of NfnB are conserved in DsrL. The carboxy-terminus of DsrL constitutes a ferredoxin domain with the principal ability for binding two additional [4Fe4S] clusters. Strep-tagged *A. vinosum* DsrL was produced in and purified from *E. coli* BL21 Δ*iscR* [5] under anoxic conditions. One liter of culture yielded 21 mg DsrL that proved to be loaded with FAD and FeS clusters upon UV/vis spectroscopic and quantitative analyses. DsrL exhibited NAD(P)H:acceptor oxidoreductase activity with a strong preference for NADH (Km=116.4 ± 17.9 µM) over NADPH (Km=288.4 ± 53 µM). Specific activity with NADH [16.0 ± 0.8 U/mg] was 3.5-fold higher compared to NADPH [4.4 ± 0.3 U/mg]. DsrL also catalyzed generation of NADH from NAD+ [Km= 123.7 ± 32.7 µM] with reduced methylviologen as electron donor with a specific activity of 16.7 ± 1.5 U/mg. The enzymatic activity, the presence and arrangement of FeS clusters and FAD, together with its similarity to NfnB and its close association with DsrAB lead us to suggest that DsrL accepts electrons from DsrAB and transfers them ultimately onto NAD+, possibly enabled by an electron-confurcating mechanism.

References
[1] Stockdreher *et al*. 2014 JBC: 289:12390
[2] Santos, Venceslau *et al*. 2015 Science 350: 1541
[3] Lübbe *et al.* 2006 FEMS Lett. 261: 194
[4] Demmer *et al*. 2015 JBC 290: 21985
[5] Kuchenreuther *et al*. 2010 PloS One 5: e15491

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

476/GMBP

Heme Ligation and Redox Chemistry in Thiosulfate Dehydrogenase (TsdA) Enyzmes

L. Jenner[1], J. Kurth[2], S. van Helmont*[2], C. Dahl[2], J. Butt[1], M. Cheesman[1], J. Bradley[1]
[1]*University of East Anglia, School of Chemistry, Norwich, United Kingdom*
[2]*Institut für Mikrobiologie und Biotechnologie, Bonn, Germany*

Thiosulfate dehydrogenase (TsdA) enzymes are a phylogenetically widespread family of periplasmic *c* type diheme cytochromes which catalyse both thiosulfate oxidation and tetrathionate reduction [1,3]. The rates of both these reactions vary between enzymes isolated from different bacteria such that the direction of the reaction most rapidly catalysed is an intrinsic property of each TsdA [4]. Currently the best characterised TsdA enzymes are from the purple sulfur bacterium *Allochromatium vinosum* [1,2] and the mammalian gut pathogen *Campylobacter jejuni* [3,4]. *Av* TsdA oxidises thiosulfate more rapidly than it reduces tetrathionate and this observation is reversed in *Cj* TsdA [3].

Av and *Cj* TsdA and several of their variants [1,4] have been characterised by Magnetic Circular Dichroism (MCD) at UV-visible and nIR wavelengths to inform on the heme ligands and redox properties in solutions of these enzymes. Previous crystallographic studies using *Av* TsdA have identified His-53 and Cys-96 as axial ligands to Heme 1 located adjacent to a putative substrate binding pocket [1,2]. This unusual heme ligation is conserved in all TsdA enzymes and nIR MCD confirms His/Cys-ligation is also present for both enzymes in solution. Variants which change this ligation such as the *Cj* TsdA C138H and C138M investigated here are catalytically incompetent suggesting this heme as the active site [1,4]. MCD shows both these variants are fully low-spin with the heme ligated by the His(Met) introduced at position 138. The TsdA Heme 2 is ligated by His-164/Lys-208 in oxidised *Av* TsdA crystals [1,2] and MCD confirms this ligation in solutions of the enzyme. However, in the sequence of *Cj* TsdA Lys-208 is replaced by the non-heme-ligating Asn-254 and Heme 2 has His/Met ligation. Oxidised Av TsdA K208N, which mimics the *Cj* TsdA sequence, displays His/Met ligation at Heme 2. However, in the oxidised *Cj* TsdA N254K variant, which mimics the environment of *Av* TsdA, Heme 2 exists as a 50:50 mix of His/Lys and His/Met ligation.

Comparison of the MCD for oxidised enzymes with those for the enzymes reduced by ascorbate ($E°'$ = -66 mV vs. SHE) and dithionite ($E°'$ = -660 mV vs. SHE) provides an indication of the potential windows in which the hemes of each protein are redox active.

References

[1] Brito *et al.* (2015) JBC 290, 9222
[2] Grabarczyk *et al.* (2015) JBC 290, 9209
[3] Kurth *et al.* (2015) JACS 137, 13232
[4] Kurth *et al.* (2016) Biosci Rep DOI: 10.1042/BSR20160457

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

477/GMBP

A novel bipartite Cupredoxin from the Anammox Bacterium *Kuenenia stuttgartiensis*

A. Dietl*[1], N. M. de Almeida[2], J. T. Keltjens[2], M. S. M. Jetten[2,3], B. Kartal[2,4], T. R. M. Barends[1]
[1]*Max Planck Institute for Medical Research, Biomolecular Mechanisms, Heidelberg, Germany*
[2]*Radboud University Nijmegen, Institute for Water and Wetland Research, Nijmegen, Netherlands*
[3]*Delft University of Technology, Department of Biotechnology, Delft, Netherlands*
[4]*Max Planck Institute for Marine Microbiology, Department of Biogeochemistry, Bremen, Germany*

Anaerobic ammonium-oxidizing (anammox) bacteria are important players in the nitrogen cycle of the Earth and are increasingly being applied in waste water treatment. The anammox metabolism is based on the combination of nitrite (NO_2^-) and ammonium (NH_4^+) to form dinitrogen gas and water *via* the highly reactive intermediates nitric oxide (NO) and hydrazine (N_2H_4) [1]. Genetic and biochemical studies of the anammox model organism *Kuenenia stuttgartiensis* revealed a broad variety of metal-containing enzymes and their associated redox partners such as cytochromes c, iron-sulfur cluster proteins and cupredoxins (CPRs). The *K. stuttgartiensis* genome encodes five paralogues of such type I (blue) copper proteins being part of the amicyanin [2] family. Strikingly, the anammox cupredoxins are about twice the size of canonical blue copper proteins with the CPR domain located in the middle of their sequence.

The cupredoxin kustd1713 was heterologously expressed in *Escherichia coli*, reconstituted with copper ions *in vitro* and a molecular model was determined at atomic resolution (1.15 Å) by X-ray crystallography. The crystal structure reveals two domains, the canonical eight-stranded β-barrel CPR domain as well as a split seven-stranded β-barrel consisting of the N- and C-terminal parts of the protein. The latter domain shows structural similarity to the C-terminal subdomain of carboxypeptidase D domain II and harbours a loop which is tethered *via* a conserved disulfide bridge to the tip of the CPR domain, close to the copper binding site. Interestingly, the typical ligand-to-metal charge transfer bands observed in the UV-Vis spectrum of common blue copper proteins in the cupric state are absent in the kustd1713 spectrum. Further EPR spectroscopic and electrochemical investigations of this unusual cupredoxin are underway.

References

[1] B. Kartal et al.; FEMS Microbiol Rev 37, 428 (2013)
[2] R. Durley et al.; Protein science 2, 739 (1993)

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

478/GMBP

The role of polyamines in growth of avian pathogenic *Escherichia coli.*

® Guerra*[1], A. Herrero-Fresno[1], J. Elmerdahl Olsen[1]
[1]*University of Copenhagen, Department of veterinary Disease Biology, Copenhagen, Denmark*

Polyamines are recognized as important requirements for eukaryotic and prokaryotic cells, present in almost all living cells, likely they came from the one universal common ancestor and could be considered as part of the cell minimal metabolism. Polyamines are necessary for cells to grow and divide properly, as they contribute to the optimal synthesis of nucleic acids and proteins used in different growth processes. The aim of this study was to provide a better understanding of why inhibition of polyamine biosynthesis and uptake system can inhibit bacteria during infection. The first step was constructed defined mutants in *E.coli O33:H4*-ST117 that blocks biosynthesis and polyamine uptake systems. The genotypes obtained are single mutations

*Δspe*B, *Δspe*C and *Δspe*F, and one double mutation *Δspe*D/*E,* responsible for the biosynthesis of putrescine and spermidine, respectively. It has also created the double mutation, combining *spe*B and *spe*C genes, that blocks part of putrescine pathway. Furthermore, single mutations in *pot*E, *yee*F, *pot*ABCD, *pot*FGHI genes were created, encoding the genes responsible for the polyamine transporters system. Bioscreen® was performed to analyze the ability of the mutant"s growth in minimal media and minimal media supplemented with putrescine and spermidine. The *Δ*potE strain, which according to the current understanding of the role of the protein PotE is unable to transport putrescine, showed an increase of lag-phase length in minimal media and minimal media supplemented by putrescine and/or spermidine. The double biosynthesis mutation *Δ*speB/C showed an increase of lag-phase length as well as delayed growth rate in minimal media. The other biosynthesis and transport mutants grew almost as well as the wild type. Ultra-High performance liquid chromatographic (UHPLC) was performed in order to quantify the amount of intracellular polyamine present in *Δ*potE and *Δspe*B/C strains. At the conference, we will highlight the features of the growth experiments and UHPLC analysis.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

479/GMBP

Structure and function of class II benzoyl-CoA reductase of anaerobic *G. metallireducens*

S. G. Huwiler[1], C. S. Seelmann*[1], C. Löffler[1], S. E. L. Anselmann[1], W. Tobias[2], B. Till[3], H. J. Stärk[4], U. Ermler[2], M. Boll[1]
[1]*University of Freiburg, Microbiology, Freiburg, Germany*
[2]*Max-Planck-Institute of Biophysics, Frankfurt, Germany*
[3]*University of Freiburg, Institute of Physical Chemistry, Freiburg, Germany*
[4]*Helmholz Centre for Environmental Research (UFZ), Analytics Department, Leizpig, Germany*

Introduction: The degradation of aromatic compounds, which include toxic and carcinogenic substances, is of central importance for the global carbon cycle. Under anaerobic conditions most of the monocyclic aromatic compounds are converted into the central intermediate benzoyl-coenzyme A (CoA). Benzoyl-CoA reductases (BCRs) dearomatize benzoyl-CoA to cyclohexa-1,5-diene-1-carboxyl-CoA, whereas two classes of BCRs are known: The ATP-dependent class I BCR of obligate anaerobic bacteria and the ATP-independent class II BCR of obligate anaerobic bacteria like in the Fe(III)-reducing γ-proteobacterium *Geobacter metallireducens*. The midpoint potential of the benzoyl-CoA/1,5-dienoyl-CoA redox pair is one of the most negative for an enzymatic reaction[1].
Objectives: The class II BCR of *G. metallireducens* was investigated to understand the structure and function of this enzyme catalysing such an extraordinary reaction.
Materials & methods: From wild type the class II BCR complex of *G. metallireducens* was purified and the cofactor content was determined. The tungsten in the active site[2] in the BamBC subunit of the class II BCR complex was investigated using EPR spectroscopy to obtain more information about the reaction mechanism at the active site.
Results: The class II BCR complex of *G. metallireducens* employs a $[Bam(BC)_2DEFGHI]_2$ subunit architecture and several FeS clusters, FADs and tungsten[3]. The tungsten in the active site interacts with a proton in the W(V+) state.
Conclusion: Results from the biochemical and spectroscopic investigations provide initial insights into the structure-/function relationship of an enzymatic electron-transfer reaction at an extremely-low redox potential.

References

[1] J.W. Kung *et al. J. Am. Chem. Soc.* 132 (2010), p. 9850-56.
[2] J.W. Kung *et al. Proc Natl Acac Sci U.S.A.* 106 (2009), p. 17687-92.
[3] Weinert & S. Huwiler *et al. Nat Chem Biol* 11 (2015), p. 586-91.
[4] C. Löffler Dissertation University of Leipzig (2012).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

480/GMBP

Molecular basis of polyamine and ethanolamine utilization in *Streptomyces coelicolor*

S. Krysenko*[1], N. Okoniewski[1], A. Kulik[1], A. Matthews[1], J. Grimpo[1], W. Wohlleben [1], A. Bera [1]
[1]*University of Tuebingen, IMIT, Microbiology and Biotechnology, Tuebingen, Germany*

Although naturally occurring polyamines (putrescine, cadaverine, spermidine and spermine) are absolutely required for cell growth, maturation and proliferation, they can be very toxic when present in excess. As strongly positive charged molecules, they can interact with DNA, RNA and with other components of the cell leading to deregulation of the cell metabolism causing lethal effect. Free living *Streptomyces sp.* and other non-motile actinobacteria have to cope with very high concentrations of polyamines that are produced during the decomposition of animal bodies and are locally released from putrefying cadaver into the soil. Ethanolamine is a component of cell membranes as phosphatidylethanolamine, which is a second-most-abundant head group of phospholipids. Both polyamines and ethanolamine can be used as nitrogen sources by *Streptomyces*. However utilization pathways of these alternative nitrogen sources for actinobacteria have not been described so far. Our study shows that the glutamine synthetase-like enzymes GlnA3 and GlnA4 are involved in the first step of the polyamine and ethanolamine utilization pathways, respectively. Based on our experimental results with the *glnA3* mutant and on comparative *in silico* analysis of a GlnA3 structure model we reasoned that GlnA3 resembles features of a gamma-glutamylpolyamine synthetase (GPS). *In vivo* experiments as well as an *in vitro* enzymatic assay showed that GlnA4 uses glutamate and ethanolamine as substrate and ATP as an energy donor generating gamma-glutamylethanolamide. Although both enzymes catalyze a glutamylation reaction, the GlnA3 and GlnA4 could not substitute each other, indicating that GlnA3 is specific for polyamine catabolism and that ethanolamine utilization requires the presence of GlnA4. These enzymes play an important physiological role ensuring both nutrients availability (*C*- and *N*-source) and resistance against high polyamine and ethanolamine concentrations. GlnA3 represents a novel promising target for a new antibiotic drug development since this kind of enzyme does not naturally occur in eukaryotes. Inhibition of GlnA3 might be an effective therapeutic strategy resulting in increased polyamine sensitivity among pathogenic actinobacteria. GlnA4 may have a high biotechnological potential in industry as a new enzyme for a glutamylation of short amines and amine alcohols.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

481/GMBP

Biochemical characterization of the putative heme chaperone HemW

T. M. Mingers*[1], V. Haskamp[1], K. Müller[1], M. Jahn[1], D. Jahn[1]
[1]*TU Braunschweig, Institute of Microbiology, Braunschweig, Germany*

Bacteria, plants and animals possess *hemW* genes annotated to encode a radical SAM-dependent Coproporphyrinogen III dehydrogenase, a potential enzyme of heme biosynthesis. We demonstrated for the Escherichia coli protein the presence of an

intact $[4Fe4S]^{2+}$ cluster and of SAM, however no radical formation or corresponding enzymatic activity was observed. Instead, covalent heme binding and heme chaperone activity was shown. HemW required the $[4Fe4S]^{2+}$ cluster for dimerization and binds 1 mol covalently bound heme per subunit. Using the *Pseudomonas aeruginosa* bacterial two hybrid system the physical interaction of HemW with bacterioferritin (BfrB), bacterial ferritin (BfrA) and the respiratory nitrate reductase (subunit NarI) was demonstrated *in vivo*. Additionally, *in vitro* studies for a direct heme transfer mediated by HemW with covalently bound hemin revealed a transmission of heme to heme-depleted quinol nitrate oxidoreductase from *E. coli* restoring enzyme activity. The heme transfer was found to depend on an intact $[4Fe4S]^{2+}$ cluster. Overall, the previous annotated radical SAM-dependent Coproporphyrinogen III dehydrogenase HemW was identified as a heme chaperone in bacteria, but is missing the obvious radical SAM-based catalysis. Therefore, we suggest to add a new class of radical SAM proteins, the non-radical forming heme chaperones.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

482/GMBP

Impact of branched-chain amino acid catabolism on fatty acid and hydrocarbon composition of *Micrococcus luteus*

M. Surger*[1], A. Angelov[1], P. Stier[1], M. Übelacker[1], W. Liebl[1]
[1]*Technical University of Munich, Microbiology, Freising, Germany*

Biosynthesized aliphatic hydrocarbons are an attractive target in microbial metabolic engineering. The reason is that these molecules can be very similar or even identical to the main components of petroleum-derived fuels, which makes them fully compatible with the existing infrastructure. Although hydrocarbon biosynthesis in bacteria has been known for decades, only recently (since 2008) have the underlying biochemical mechanisms begun to be elucidated. One of the routes, described initially in *Micrococcus luteus*, involves the head-to-head condensation of two fatty acid intermediates leading to long-chain (C23 - C33) olefins with the double bond near the centre of the molecule. The genes encoding the enzymes of this pathway, termed *ole*ABCD, were first identified in *M. luteus*, but homologs have been later found in many other bacteria. Because fatty acyl chain length, degree of saturation and branching are key to the properties of the derived hydrocarbons, the understanding of the mechanisms that modulate the fatty acid synthesis is critical for the ability to design fuels with defined structures. The olefins produced by *M. luteus* are terminally (*iso*- or *anteiso*-) methyl-branched, and it is presumed that the type of branching depends primarily on the availability of short and branched chain acyl-CoA primers, intermediates of the branched-chain amino acid (BCAA) metabolism. In this report, we demonstrate the key role of the branched-chain amino acid metabolism in *M. luteus* for the branching pattern of the produced olefins. We identify in *M. luteus* the genes encoding the branched-chain alpha-keto acid decarboxylase, which as major player of the branched-chain fatty acid synthesizing system is actually producing the fatty acid primer molecules. We identify the genes encoding the short branched-chain acyl-CoA dehydrogenases, involved in the individual degradation of the primer molecules, which arise either from isoleucine, leucine or valine. Finally, we show how, by manipulating the genes of individual BCAA pathways, custom branching patterns of the micrococcal olefins can be achieved.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

483/GMBP

Pathometabolism of intracellular bacteria: the power and limits of isotopologue profiling

W. Eisenreich*[1], C. Huber[1], I. Häuslein[1]
[1]*TU München, Garching, Germany*

Metabolic adaptation is a key feature of pathogenic bacteria interacting with host organisms, especially when these pathogens replicate inside their host cells. As a result of this adaptation process, the metabolic pathways and fluxes in the pathogen, but also in the host are modulated to the benefit or disfavor for the partners. For this complex metabolic interplay, the term pathometabolism has been coined by Werner Goebel. There are now plenty of examples that intracellular pathogens like *Listeria monocytogenes*, *Legionella pneumophila*, *Coxiella burnetii*, *Francisella tularensis*, *Chlamydia trachomatis* or *Shigella flexneri* specifically adapt their nutrient usages, the metabolic pathways and fluxes to the various environments encountered during their infection processes. On the other hand, the metabolic processes of the respective host cells also seem to be changed during infections with these bacteria, when the pathogens efficiently retrieve host nutrients during their replication. However, the knowledge of metabolic adaptation during bacterial infections is still limited. This is unsatisfactory since metabolic adaptation is central to understand bacterial infections in general on a molecular/metabolic level. Moreover, it can be expected that many hitherto unexplored bacterial and host cell targets are among the components involved in pathometabolism.
A method to analyze metabolic pathways and fluxes under complex and physiological conditions is based on incorporation experiments with infected cells or organisms using ^{13}C-labeled substrates. ^{13}C-Enrichments and positional isotope distributions in multiple metabolic products from the bacteria and the hosts can be analyzed by mass spectrometry and /or NMR spectroscopy providing detailed quantitative information about substrate uptake and usage, the activity and adaptation of target reactions or whole pathways under study, or the impacts of further environmental factors upon these processes.
Isotopologue studies of the authors in cooperation with microbiologists on the pathometabolism of intracellular bacteria reflect a bipartite structured metabolism with multiple substrate usages of the pathogens as a common motif. A comparison of non-infected vs. infected host cells shows the impacts of infection upon host metabolism. The power and limits of isotopologue profiling in studying metabolism of complex biological systems will be presented with recent examples.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

484/GMBP

Conversion of cysteine to 3-mercaptopyruvic acid by bacterial aminotransferases

C. Andreeßen*[1], V. Gerlt[1], A. Steinbüchel[1]
[1]*Westfälische Wilhelms-Universität Münster, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*

3-Mercaptopyruvate (3MPy), a structural analog of 3-mercaptopropionic acid (3MP), is a precursor compound for biosynthesis of polythioesters in bacteria. The cost effectiveness and sustainability of the whole process could be greatly improved by using the cysteine degradation pathway for an intracellular supply of 3MPy. Transamination of cysteine to its corresponding α-keto acid 3MPy is catalyzed by cysteine aminotransferases (CAT). However, CAT activity has so far not been described for bacterial aminotransferases (AT), and it was unknown whether they can be applied for the conversion of cysteine to 3MPy. In this study, we selected eight bacterial AT based on sequence homology to CAT of *Rattus norvegicus* (Got1). The AT included four aspartate aminotransferases (AATs) and four aromatic amino

acid aminotransferases (ArATs) from *Advenella mimigardefordensis* DPN7, *Escherichia coli* MG1655, *Shimwellia blattae* ATCC 33430, *Ralstonia eutropha* H16 and *Paracoccus denitrificans* PD1222. For a more detailed characterization, all selected AAT or ArAT encoding genes were heterologously expressed in *E. coli* and purified to electrophoretic homogeneity using nickel affinity chromatography. CAT activity was detected for all AT when a novel continuous coupled enzyme assay was applied. Kinetic studies revealed the highest catalytic efficiency of 5.1 mM/s for AAT from *A. mimigardefordensis*. Formation of 3MPy from cysteine could additionally be verified by an optimized approach using derivatization of 3MPy with the Girard T reagent and liquid chromatography-mass spectrometry analyses. The introduction of AAT from *A. mimigardefordensis* into *E. coli* already results in a potential production strain for the bioconversion of cysteine to the much more valuable product 3MPy, thus achieving a significant upgrading. Another promising approach would be the establishment of a biosynthetic pathway to convert cysteine to 3MP via 3MPy which would enable PTE biosynthesis based on cysteine instead of expensive organic sulfur compounds which have currently to be applied. Hence, on the one hand, the presence of significant cysteine transamination activity, which could be described for the first time for bacterial aminotransferases in this study, is of academic interest, as it reveals the initial step in the degradation pathway for cysteine in bacteria. On the other hand, the findings presented here, also entail a great potential for future biotechnical applications.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

485/GMBP

Effect of NaCl concentration and amino acid supplementation on the growth of *Clostridium acetobutylicum*

M. Lehmann*[1], H. Bahl[1], R. J. Fischer[1]
[1]*University of Rostock, Microbiology, Rostock, Germany*

The Gram-positive bacterium *Clostridium acetobutylicum* is well known due to its ability to produce solvents like butanol, a next-generation biofuel. As a soil-dwelling microorganism, this strict anaerobic bacterium lives under ever-changing environmental conditions including periods of salt stress. A common adaption strategy to cope with high salt concentrations is the accumulation of compatible solutes, e.g. amino acids. Here, we show the effect of amino acid supplementation on growth of *C. acetobutylicum* ATCC 824 exposed to NaCl stress to determine whether amino acids are involved in the salt adaption strategy of this bacterium. Growth experiments in minimal media revealed that increasing sodium chloride concentrations led to the reduction of biomass and glucose consumption. Additionally, a decreased solvent production could be observed. The supplementation of NaCl-containing media with glycine betaine and proline showed a positive effect on the biomass production of *C. acetobutylicum*, whereas arginine, histidine and leucine led to a higher production of solvents. Thus, we demonstrate, that amino acids play an important role within in the adaptation strategy of *C. acetobutylicum* against salt stress.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

486/GMBP

Identification of assembly factors for the cytochrome bc_1-aa_3 supercomplex in *Corynebacterium glutamicum*

X. Schuplezow[1], C. Davoudi*[1], M. Brocker[1], M. Baumgart[1], M. Bott[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Institute of Bio- and Geosciences, Jülich, Germany*

Introduction: *Corynebacterium glutamicum* possesses a branched respiratory chain with two terminal oxidases, cytochrome aa_3 and cytochrome *bd* (1). Cytochrome aa_3 forms a supercomplex with the cytochrome bc_1 complex. Cytochrome c_1 contains two covalently bound heme groups and is the only *c*-type cytochrome present in *C. glutamicum* (2,3). Recent studies suggested that these features are typical for the majority of Actinobacteria (4). The absence of a functional bc_1-aa_3 supercomplex results in a strong growth defect (2,3,5). As the cytochrome aa_3 oxidase is a copper-dependent enzyme, copper sufficiency and effective copper insertion are prerequisites for its functionality. However, the pathway of copper insertion is completely unknown yet.
Objectives: The aim of this work was the identification of accessory proteins required for the formation of a functional bc_1-aa_3 supercomplex by analyzing *C. glutamicum's* global response to copper deficiency, which also complements our previous studies on copper excess stress (6) and thus contributes to our understanding of copper homeostasis.
Material and Methods: To analyze the global transcriptional response to copper deficiency, we compared cells cultivated under copper limitation and copper sufficiency using DNA microarrays. Selected target genes showing increased expression under copper deficiency were deleted and growth of the resulting mutants was monitored. Formation of the bc_1-aa_3 supercomplex in the mutants was analyzed by co-purification experiments.
Results: Transcriptome analysis revealed a limited number of genes with elevated expression levels under copper limitation. Bioinformatic analysis of the corresponding proteins suggested that some of them might be involved in copper transport or insertion into cytochrome aa_3. Deletion studies of selected genes revealed two candidates, *ctiP* and *copC,* whose absence caused strong growth defects under standard copper levels. The *ctiP* gene encodes a 79 kDa membrane protein and its deletion caused an increased copper resistance and failed to form a stable bc_1-aa_3 supercomplex. The *copC* gene encodes a 21 kDa periplasmic protein anchored to the membrane by a single transmembrane helix. Similar to *ctiP*, absence of *copC* led to an increased copper resistance and prevented formation of a stable bc_1-aa_3 supercomplex.
Conclusion: The results of this work suggest that both CtiP and CopC play a role in copper homeostasis and copper insertion into the bc_1-aa_3 supercomplex and allow studies on the molecular details of this process.

References

[1] Bott, M. and Niebisch, A. (2003) J. Biotechnol. 104, 129-153
[2] Niebisch, A. and Bott, M. (2001) Arch. Microbiol. 175, 282–294
[3] Niebisch, A. and Bott, M. (2003) J. Biol. Chem. 278, 4339–4346
[4] Kao, W. et al. (2016) Biochim. Biophys. Acta 1857, 1705–1714
[5] Koch-Koerfges et al. (2013) Biochim. Biophys. Acta 1827, 699–708
[6] Schelder, S. et al. (2011) PLoS ONE 6(7), e22143

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

487/GMBP

The role of *Pseudomonas aeruginosa* clone C-specific genomic island-1 (PACGI-1) in protein quality control and heat tolerance

C. Lee*[1], S. M. Kamal[1], H. Kim[1], H. Lünsdorf[2], M. Nimtz[2], L. Jänsch[2], U. Curth[3], B. Bukau[4,5], A. Mogk[4,5], U. Römling[1]
[1]*Karolinska Institutet, Department of Microbiology, Tumor and Cell Biology, Stockholm, Sweden*
[2]*The Helmholtz Center for Infection Research, Braunschweig, Germany*
[3]*Hannover Medical School, Institute for Biophysical Chemistry, Hannover, Germany*
[4]*University of Heidelberg (ZMBH), Center for Molecular Biology , Heidelberg, Germany*
[5]*DKFZ-ZMBH Alliance Heidelberg, German Cancer Research Center (DKFZ), Heidelberg, Germany*

Pseudomonas aeruginosa is the prototype of a highly successful nosocomial pathogen capable to cause a wide variety of infections. *P. aeruginosa* clone C strains are most prevalent occurring in clinical and environmental aquatic habitats and causing infections in patients. Factors determining the success of clone C strains in host infection and environmental transmission and survival are largely unknown. Previously, investigation of a collection of clone C strains from diverse clinical and environmental habitats identified an unusually high flexibility in the clone C strain pan-genome with genomic arrangements such as acquisition and deletion of genomic islands comprising more than 10% of the genome. Recently, whole-genome sequencing, comparative genome analysis and protein expression studies led to the identification of the genomic island-1 (PACGI-1) as clone C hallmark. Interestingly, PACGI-1 contains a number of genes involved in protein homeostasis located on a transmissible locus for protein quality control-1 (TLPQC-1). Deletion of PACGI-1 showed this genomic island to be associated with elevated heat tolerance, potentially providing a competitive growth advantage in natural and host environments. Here, we focus on the molecular mechanism of heat tolerance provided by the *shsp20GI-clpGGI* operon in PACGI-1. sHsp20GI belongs to the family of small heat shock proteins, associating with misfolded proteins and preventing their aggregation. ClpGGI constitutes a novel and so far uncharacterized member of Hsp100 chaperones, linked to protein disaggregation. We purified and characterized the basic chaperone activities of sHsp20GI and ClpGGI. sHsp20GI exhibits classical chaperone activity, while GlpGGI is highly efficient in rescuing proteins from an aggregated state. GlpGGI disaggregation activity is extraordinary high, qualifying it the most potent disaggregase reported to date. We also constructed deletion mutants of *clpGGI* and *dna-shsp20GI-clpGGI* demonstrating important contribution to heat shock resistance. In summary, we conclude that the protein quality control genes on PACGI-1 are involved in heat tolerance, which is assumed to contribute to successful survival and spread of worldwide distributed clone C strains.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Genomics and Metagenomics (FG GMG)

488/GMGP

Comparative analysis of genome properties and mechanisms of niche adaptation within the marine *Octadecabacter* genus

F. Lenk*[1], R. Daniel[1], A. Leimbach[2], J. Vollmers[3]
[1]*Institute of Microbiology and Genetics, Genomic and Applied Microbiology, Göttingen, Germany*
[2]*Instute for Hygiene, Münster, Germany*
[3]*Leibniz Institute DSMZ, Braunschweig, Germany*

Introduction: Members of the genus *Octadecabacter* are Gram-negative and thrive in different marine habitats. The first two species (*O. arcticus* and *O. antarcticus*) were isolated from sea ice (Gosink et al. 1997). Analysis of their genomes revealed the presence of a specific type of microbial rhodopsin and high genome plasticity, mediated through a high number of transposable genetic elements (Vollmers et al. 2013). Both species are strictly psychrophilic and share almost identical 16S rRNA gene sequences, but show a bipolar distribution (Arctic vs. Antarctic). Recently, further *Octadecabacter* strains from temperate zones were isolated, cultured and sequenced.
Objectives: The current nine *Octadecabacter* genomes warrant a closer look into the genomic composition of this genus. Since polar and temperate isolates persist in different environments, we aimed to analyze their gene content and sequence composition in relation to their lifestyles. This should provide insights into the adaptation mechanisms for conditions prevalent at their respective habitats.
Materials & Methods: We sequenced the genomes of seven cultured *Octadecabacter* strains with Illumina high-throughput sequencing techniques. These genomes plus references were used for comparative genomic analyses and reconstruction of phylogenetic relationships within this genus.
Results: Although all isolates showed high similarity in their rRNA gene sequences, whole genome phylogeny exposed considerable differences in shared gene content, as well as sequence composition, leading to the formation of different clades within the *Octadecabacter* genus. Interestingly, the psychrophilic isolates form a separate cluster. We could identify several genetic regions which are specific for different habitats and environmental parameters, such as salinity and nutrient availability and thus contribute to habitat adaptation. Likewise, we observed differences in sequence composition, which we could attribute to psychrophilic adaptation of the polar cluster of *Octadecabacters*.
Conclusion: The habitat separation of different *Octadecabacter* isolates has a strong impact on their gene content and composition. With our analyses, we could attribute a large part of the observed differences to specific niche adaptation and identify factors, which led to the formation of different subclades within the *Octadecabacter* genus.

References

[1] Gosink, J.J., Herwig, R.P. & Staley, J.T., 1997. Octadecabacter arcticus gen. nov., sp. nov., and O. antarcticus, sp. nov., Nonpigmented, Psychrophilic Gas Vacuolate Bacteria from Polar Sea Ice and Water. *Systematic and Applied Microbiology*, 20(3), pp.356–365.
[2] Vollmers, J. et al., 2013. Poles apart: Arctic and Antarctic Octadecabacter strains share high genome plasticity and a new type of xanthorhodopsin. *PloS one*, 8(5), p.e63422.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

489/GMGP
Identification of three novel metagenomic-derived lipolytic enzymes from Azorean hot springs
P. Busch*[1], U. Lorenz[1], S. Elleuche[1], G. Antranikian[1]
[1]*Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany*

Introduction: Lipolytic enzymes containing esterases (EC 3.1.1.1) and lipases (EC 3.1.1.3) are essential for the cleavage and formation of ester bonds from triacylglycerides [1,2]. Due to their ability to perform hydrolysis, transesterification and glycerolysis as well as their enantio- and stereoselectivity, this group of hydrolases offers great biotechnological potential for many industrial applications.
Extremophilic microorganisms are suitable sources to identify new enzymes, which can function under the harsh conditions of biotechnological processes [3]. In order to use this potential, metagenomic approaches have been applied and optimized during the last decades to investigate the genetic information of many non-cultivatable microorganisms.
Objectives: In this study, a metagenomic approach was used to identify novel lipolytic enzymes from Azorean hot springs with regards to possible biotechnological applications [4].
Materials & methods: Isolated DNA from Azorean hot springs was sequenced by 454 pyrosequencing and the metagenomic dataset was screened for putative esterases and lipases. Genes were amplified and cloned, the produced proteins were purified and enzyme characterization was performed.
Results: The characterization of AzEst03, AzEst06 and AzEst08 yielded activity at elevated temperatures (60-70 °C), at alkaline pH (8.0-9.0) and a diverse substrate specificity. An increase of the relative activity was observed after incubating the enzymes in organic solutions.
Conclusion: New lipolytic enzymes from extremophiles were identified by a metagenomic approach. Three genes, *azEst03*, *azEst06* and *azEst08* encoding putative esterases were cloned, recombinantly produced in *E. coli* and the purified enzymes were characterized. All enzymes are promising candidates for various biotechnological processes due to their catalytic activity in organic solvents.

References
[1] J L Arpigny & K E Jaeger, Biochemical Journal, 343 (1999) 177
[2] U T Bornscheuer, FEMS Microbiology Reviews, 26 (2002) 73
[3] S Elleuche *et al.*, Biotech. of Extremophiles, Ch. 12
[4] S Sahm *et al.*, Extremophiles, 17 (2013) 649

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

490/GMGP
Combining metagenomic with compositional analysis to elucidate the complex microbiome of an anaerobic digester
D. Taraba*[1], F. Bonk[1], D. Popp[1], H. Harms[1], F. Centler[1]
[1]*Helmholtz Centre for Environmental Research - UFZ, Environmental Microbiology , Leipzig, Germany*

The phases of anaerobic digestion leading to the formation of biogas as an important renewable energy source are largely understood. However, low methane yields and process instabilities are a constant threat to an efficient operation of biogas plants. The aim of this project is to investigate the diversity and activity of, and interactions within microbial biogas communities to build predictive models to better understand community dynamics. As a starting point, we compare two reactors operated under alternative feeding regimes. In order to reduce the complexity of the microbial communities only the last two steps of anaerobic digestion were implemented in the reactors.
Two continuously stirred tank reactors digesting short chain fatty acids (acetate, propionate and butyrate with a COD based ratio of 0.45:0.1:0.45) were operated under mesophilic conditions. One reactor was fed continuously whereas the other reactor was fed discontinuously (75% of the substrate as a daily pulse and the remaining 25% continuously). The bacterial communities of both reactors were studied by amplicon sequencing of the 16S rRNA genes using the 454-pyrosequencing platform GS Junior (Roche). The methanogenic communities were investigated by the molecular fingerprinting technique T-RFLP (terminal restriction fragment length polymorphism) based on the methyl coenzyme M reductase (*mcrA*) genes.
Despite the altered feeding strategy the methane yield was nearly equal for both reactors (0.25 LN CH_4 $gCOD^{-1}$). The bacterial communities had a surprisingly high diversity (180 OTUs) even though the anaerobic digestion process was restricted to acetogenesis and methanogenesis only. Differences in community abundances between both reactors were observed. Syntrophic butyrate degrading species of the genus *Syntrophomonas* were found in higher relative abundance in the discontinuously fed reactor (10.5%) compared to the continuously fed reactor (2.0%). Furthermore, syntrophic propionate-oxidizing species of the genus *Pelotomaculum* had a higher abundance in the continuously fed reactor (1.1%) compared to the discontinuously fed reactor (0.1%). Members of the species *Methanoculleus* and *Methanosaeta* were found with similar abundances in both reactors (35% and 32% for *Methanoculleus,* and 54% and 52% for *Methanosaeta*). In contrast, *Methanosarcina* was only found in the discontinuously fed reactor (7 %).
The microbial communities of both reactors were similar. Variations were observed mainly for the relative abundances of butyrate and propionate degrading species and also for *Methanosarcina*. Hence, the feeding strategy affected the relative abundance of bacterial and methanogenic communities. To additionally detect functional differences, metagenomic data is analyzed, and will later be accompanied by metatranscriptomics and metaproteomics to elucidate differences in activity.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

491/GMGP
***E. coli* functional genotyping: predicting phenotypic traits from whole genome sequences**
L. Hauben[1], D. De Coninck*[1], K. Vranckx[1], K. De Bruyne[1]
[1]*Applied Maths NV, Sales & Support, Sint-Martens-Latem, Belgium*

Introduction: Foodborne bacteria like *E. coli* pose a major threat to public health. To prevent widespread infections due to these bacteria, as well as to detect outbreaks, rapid and accurate identification and characterization of these bacteria are of key importance.
Objectives: Conventional methods for serotyping, virulence profiling, and antimicrobial susceptibility testing are time-consuming and often require complex workflows. Alternatively, whole genome sequence (WGS) data provide highly detailed genotypic information and could have the potential to replace some of these conventional methods. In this study, we present a genotyping plugin for the BioNumerics® 7.6 software, that predicts O and H serogroups and pathotype, and detects virulence and resistance genes as well as prophages and plasmids, starting from *E. coli* WGS data.
Materials & Methods: The BioNumerics® genotyping plugin contains public databases for e.g. serotype, virulence and resistance prediction downloaded from the Center for Genomic Epidemiology (CGE; www.genomicepidemiology.org), combined with private knowledge. The various *E. coli* genotyping tools start from the assembled genomic sequences and use a blast-based approach to detect and identify the genes of interest.

Results: Publically available sequences or sequence reads (after assembly) of isolates with known resistance and/or virulence were analysed by the BioNumerics® *E. coli* genotyping plugin. The genotyping tools were evaluated versus both traditional- and typing information obtained through the CGE tools. Typing results were recorded both as database information fields and/or phenotypic datasets. In addition, exploratory reports on the detailed genotyping results, including locus name, similarity score, and descriptive information on the detected genes, are easily made available from within the software.
Conclusion: The prediction of phenotypic traits from WGS can greatly improve the efficiency and effectiveness of molecular surveillance. The BioNumerics® functional genotyping plugin proofs to be an effective predictive tool for *E. coli* phenotypes, including serotype, antibiotic resistance, and virulence. This user friendly WGS-workflow combined with the integrated read quality assessment, wgMLST and wgSNP tools of BioNumerics®, can lead to more efficient outbreak characterization. Moreover, this tool can be adapted to other bacterial pathogens in the future.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

492/GMGP
Genomic Analyses of *Francisella tularensis* subsp. *holarctica* strain 08T0073 isolated from a wild European hare
A. Busch*[1,2], H. Tomaso[1,2]
[1]*Friedrich-Loeffler-Institut, Jena, Germany*
[2]*Friedrich-Loeffler-Institut, IBIZ, Jena, Germany*

Question: *Francisella tularensis* is a small, highly virulent, Gram-negative bacterial pathogen and the causative agent of tularemia. A genomic survey has been conducted to establish a genotyping procedure based on whole genome data of *F. tularensis.*
Method: Isolates of *F. tularensis* subsp. *holarctica* sampled from wildlife in Germany were cultured and DNA was isolated. The samples were sequenced and subjected to bioinformatic analyses. The evaluation included a comparison of assemblers (such as SPAdes, MaSuRCA, CLC workbench (Qiagen), geneiousg) and a comparison of the annotation tools RAST and Blast2Go.
Results: As result of the comparison, the assemblers Spades, MaSuRCA and the CLC workbench (Qiagen) showed approximately the same contig numbers, N50 values and estimated genome sizes, whereas Geneiousg showed less favorable quality parameters. With the help of this evaluation, a high quality draft genome sequence of a *Franscisella tularensis* subsp. *holarctica* (strain 08T0073) isolated from a wild European hare could be generated. The best results were obtained using CLC workbench which lead to a genome assembly with 97 contigs and a N50 value of 27460 bp. The final assembled genome consisted of 1775487 bp with a GC content of 32.2%. Annotation features included 1949 genes and 38 RNAs.
Conclusions: The evaluation of bioinformatic tools is a necessary step for genotyping. NGS data could be used as an important tool for the surveillance of *Francisella tularensis* subsp. *holarctica* in wildlife to monitor this zoonotic disease in the future.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

493/GMGP
Impact of database choice on taxonomic assignment results of 16S rRNA amplicon sequencing data
D. Popp*[1], D. Taraba[1], F. Bonk[1], F. Centler[1]
[1]*Helmholtz Centre for Environmental Research, Leipzig, Germany*

Next-generation amplicon sequencing of 16S rRNA genes has become an indispensable tool for the analysis of complex microbial communities. Besides experimental design, the choice of parameters during downstream analysis of raw sequences is critical for high quality results. Analysis pipelines come with default databases (e.g. Mothur pipeline with the RDP database and QIIME pipeline with the Greengenes database) which are typically adopted by the user for taxonomic assignments. However, alternative databases can be used as well and the choice of database might influence the taxonomic assignment as they differ in quality and number of sequences. Here, a 16S rRNA genes based amplicon sequencing data set was evaluated based on four different databases and results compared.
An anaerobic digester microbiome which features a complex community structure was analyzed using the 454-pyrosequencing platform GS Junior (Roche). Raw sequences were analyzed using the QIIME pipeline (version 1.9.0) with default parameters. Taxonomic assignments were based on a RDP database (trainset 14 containing 10,534 sequences), the Greengenes database (version 13.8 containing 99,322 sequences) and two SILVA-based databases. One SILVA database was the default dataset distributed for the Mothur pipeline containing 14,956 sequences and the other one the manually curated MiDAS database (version 2.1 containing 453,045 sequences).
A comparison of the results based on the four different databases revealed only minor differences on phylum level with *Firmicutes* and *Bacteroidetes* being dominant with around 44% and 35% relative abundance, respectively. Whereas more than 97% of all sequences could be taxonomically assigned on phylum level using of the SILVA-based or Greengenes databases, only 92% of the sequences could be assigned using the RDP database. In contrast to the phylum level, striking differences of the taxonomic assignments were observed on lower taxonomic levels. Most differences occured within the class *Bacteroidia* and within the order *Clostridiales*. Highest sequence assignment success on genus level (74%) could be achieved by using the MiDAS-SILVA database. Using the other databases only 23% to 37% of all sequences could be assigned.
The quality of amplicon sequencing results strongly depended on the choice of the database. Using the MiDAS-SILVA database containing by far the most sequences resulted in lowest percentage of unclassified sequences. However, the computational effort increased threefold using this database compared to the second largest Greengenes database. Further improvement of taxonomic assignment might be achieved by trimming the database sequences according to the amplified region of the 16S rRNA genes to minimize the possibility of false positive assignments.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

494/GMGP
Characterization of a novel metagenome-derived ß-glucanase with an unusual module architecture
A. Angelov*[1], V. T. T. Pham[1], S. Brady[2], B. Leis[1], M. Mörch[1], W. Liebl[1]
[1]*Technische Universität München, Lehrstuhl für Mikrobiologie, Freising, Germany*
[2]*Georg-August-Universität Göttingen, Göttingen, Germany*

Functional screening of a metagenomic library from a volcano soil sample revealed a novel thermostable β-glucanase (EngU) which exhibits unusual domain architecture and unexpected hydrolytic activities. Three regions of EngU showed amino acid sequence similarity with regions of the glycoside hydrolase (GH) families 5

and 42, placing this enzyme in the GH-A clan of glycoside hydrolases, in which the core structure is an eightfold β/α barrel. In addition, a carbohydrate-binding module (CBM4) was found, unusually, as an insertion between two regions with similarity to GH42. The recombinantly expressed enzyme hydrolyzed mixed linkage (β-1,3-β-1,4) glucans such as barley β-glucan and lichenin, and to a lesser extent also β-1,4-cellulosic substrates, such as carboxymethylcellulose and hydroxyethylcellulose. Contrarily, substrates typical for GH42 enzymes, such as lactose and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside were not cleaved, clearly showing that EngU is a glucanase rather than a β-galactosidase. Various modified versions of this protein (truncated versions and site-directed mutagenesis variants) were generated and expressed in *Escherichia coli* for a systematic characterization of the catalytic and binding modules as well as of the C-terminal part, which so far was found only in EngU homologs. Characterization of the variants revealed: (i) the C-terminus is needed for expression of an active enzyme; (ii) the residues E239 and E581 are important for activity and are presumably the catalytic nucleophile and acid/base residues, respectively; (iii) two separately expressed parts of the protein, each catalytically inactive, were able to complement each other *in vivo* and lead to an active enzyme.
In conclusion, the substrate preference and endo-activity of EngU could not be predicted from its unique hybrid GH module composition. Furthermore, and highly unusual, EngU included a complete CBM module inserted into the catalytic module between its catalytic residues. Analysis of the predicted secondary structure and expression of truncated enzyme variants suggest that the CBM module splits this (β/α)8 barrel protein in two half-barrels, which assemble to a catalytically active enzyme.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

495/GMGP

Reliable indel detection from short read bacterial genome sequencing data

M. Steglich*[1], U. Nübel[1,2]
[1]*Leibniz-Institut DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany*
[2]*German Center for Infection Research (DZIF), Partner Site Hannover-Braunschweig, Germany*

Intoduction: Short-term evolution in bacteria proceeds fast enough to enable monitoring of bacterial spatial spread on the basis of core genome variation [1]. While the detection of SNP variation is straightforward, however, the reliable detection of structural genomic variation (including insertions, deletions, and the gain and loss of genetic material) on the basis of Illumina short-read sequencing data is more difficult and, consequently, its evolutionary dynamics is less well understood. Most software tools for detection of indels are limited to particular lengths or types of variant.
Objectives and Methods: We tested and compared the performance of four indel detection applications: VarScan2 [2], Pindel [3], FreeBayes [4] and ScanIndel [5]. We analysed a simulated read dataset for a 300-kilobase genomic fragment from *Clostridium difficile* (*R20291*) and used BWA-MEM [6] for read mapping.
Results: Only ScanIndel detected indels over the entire length spectrum (1-2,300 bp) with high specificity and sensitivity. All other tested products failed to detect large proportions of long indels (I. e., indel length > 0.3 x read length), even at high sequencing coverage (> 100x fold).
Conclusion: Indel detection from short read data is still challenging, as detection capabilities varied widely among different software packages. ScanIndel performed best, as it enabled the detection of indels over the full length spectrum tested, at high specificity and sensitivity.

References

[1] Steglich, M. et al. PloS One (2015) 10 (10): e0139811. DOI: 10.1371/journal.pone.0139811
[2] Koboldt, D. et al. Genome Res. (2012) 22 (3): 568-76. DOI: 10.1101/gr.129684.111
[3] Ye, K. et al. Bioinformatics (2009) 25 (21): 2865-2871. DOI: 10.1093/bioinformatics/btp394.
[4] Garrison, E. et al. preprint at arxiv.org (2012) 9 pages, partial draft. arXiv:1207.3907v2 [q-bio.GN]
[5] Yang, R. et al. Genome Medicine (2015) 7: 127. DOI: 10.1186/s13073-015-0251-2
[6] Li, H. preprint at arxiv.org (2013) arXiv:1303.3997v2 [q-bio.GN]

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

496/GMGP

Genome characterisation of *mcr*-1 harbouring *E. coli* from livestock and food

J. A. Hammerl*[1], A. Irrgang[1], M. Grobbel[1], B. A. Tenhagen[1], S. Schmoger[1], T. Skladnikiewicz-Ziemer[1], K. Thomas[1], B. Lesniewsky[1], N. Dinse[1], A. Kaesbohrer[1]
[1]*German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany*

Question: In 2015 the first plasmid-encoded colistin resistance gene *mcr*-1 was detected in livestock, raw meat and human beings in China. Thereafter, several studies revealed a worldwide distribution of this resistance gene in different genera of the Enterobacteriaceae recovered from the environment, food, livestock, infected patients as well as asymptomatic human carriers. As colistin is a last choice antibiotic, spreading of the *mcr*-1 mediated colistin resistance might be problematic for therapeutic issues in the future. In this study we provide an overview on the genomic composition of different *mcr*-1 harbouring *E. coli* from livestock and food samples that were recovered between 2010 and 2015 in Germany.
Methods: Isolates analysed in this study originated from the German national zoonoses monitoring that allows resistance determination in commensal *E. coli* isolated from animal and food samples of animal origin by broth microdilution method. Out of 10,600 screened *E. coli*, 505 isolates from poultry, pig and cattle production chain were phenotypically determined to be colistin resistant based on a cut-off value of MIC>2 mg/l. Molecular identification by real-time PCR showed, that around 78% of the isolates harboured the *mcr*-1 gene. Whole genome sequencing was done on 24 of these isolates on an Illumina MiSeq benchtop sequencer, reflecting the different sources considered.
Results: Bioinformatical analysis revealed that a broad spectrum of commensal *E. coli* comprising different MLST-, phylo-, and serotypes harbour the *mcr*-1 resistance gene. All strains exhibit distinct patterns of virulence, antibiotic resistance genes and/or mobile genetic elements (i.e. prophages, plasmids). Interestingly, only three different *mcr*-1 plasmid variants were identified. The occurrence of the different plasmid variants could not be attributed to a specific time period, source, or serotype. The prevailing data showed that the spread of *mcr*-1 harbouring plasmids might not be associated with different *E. coli* MLST-, phylo- or serotypes but may be driven by the susceptibility of the strains for the self-transmissible plasmid and antibiotic selection pressure. Therefore, the question arises, which evolutionary events occurred for the appearance of the different *mcr*-1 encoding plasmid variants.
Conclusion: The *mcr*-1 resistance gene is widely distributed among commensal *E. coli* from different sources and time periods. However, in the sequenced *mcr*-1 positive isolates only three different plasmid variants were identified. The likelihood of plasmid transfer might be associated with the susceptibility of the strains for the *mcr*-1 encoding plasmids and the antibiotic

selection pressure. In the future, *mcr*-1 prevalence should be carefully monitored and epidemiological data from other countries are urgently needed to determine the predominant plasmid variants, evolution of these plasmids and its transferability.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

497/GMGP

Analysis of the host-pathogen interaction between *Staphylococcus aureus* and human HBE cells by dual RNA-Seq

K. Förstner*[1], H. Pförtner[2], A. Westermann[3], U. Mäder[2], U. Völker[2], J. Vogel[3]
[1]*Core Unit Systems Medicine, IMIB, Medical Deparment, Würzburg, Germany*
[2]*Interfaculty Institute for Genetics and Functional Genomics, Department of Functional Genomics, Greifswald, Germany*
[3]*Institute for Molecular Infection Biology (IMIB), Medical Department, Würzburg, Germany*

Dual-RNA-Seq is a powerful method to globally measure gene expression in pathogens and their hosts in parallel [1]. In this approach RNA of both organisms is extracted without previous separation but the allocation of sequencing reads is done computationally after combined library preparation and high-throughput sequencing. In the present study we applied this protocol to shed light on the host-pathogen interaction during the infection of cells of the human line 16HBE14o- bronchial epithelial cells by *S. aureus* HG001. *S. aureus* is one of the early pathogens that can be found in cystic fibrosis patients. In order to distinguish between infected and non-infected S9 cells the *S. aureus* strain constitutively expresses GFP and the HBE cells were separated based on their fluorescence status using FACS. Comparing the gene expression levels of *S. aureus* at different time points after infection we observed that several bacterial small RNAs with so far unknown functions are strongly differently expressed which indicates an involvement in the regulation of the infection process. Also the changes in the host transcriptome could be captured successfully and are currently studied in more depth.

References

[1] Westermann AJ, Förstner KU, Amman F, Barquist L, Chao Y, Schulte LN, Müller L, Reinhardt R, Stadler PF, Vogel J. Dual RNA-seq unveils noncoding RNA functions in host-pathogen interactions. Nature. 2016 Jan 28;529(7587):496-501. DOI:10.1038/nature16547

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

498/GMGP

Genomic adaptation of *Subtercola* sp. strain DB165 to its harsh environment in Llullaillaco Lake

A. Villalobos*[1], J. Wiese[1], H. Horn[1], P. Aguilar[2], C. Dorador[2], U. Hentschel-Humeida[1], J. F. Imhoff[1]
[1]*GEOMAR Helmholtz Centre for Ocean Research Kiel, Marine Microbiology, Kiel, Germany*
[2]*Laboratorio de Complejidad Microbiana y Ecología , Departamento de Biotecnología, Universidad de Antofagasta, Antofagasta, Chile*

Introduction: Llullaillaco is the second highest volcano on Earth (6739 m asl). It is characterized as oligotrophic environment with low precipitation, high rates of evaporation, a strong day/night fluctuation of temperatures (-14 ºC to 56 ºC), and strong UV incidence. Actinobacteria appear especially well adapted to survive under these harsh conditions and represent the most abundant phylum in Llullaillaco.
Objectives: In order to understand the genomic basis of adaptation to the extreme environmental conditions, the genome sequence of *Subtercola* sp. strain DB165, a psychrophilic Actinobacterium isolated from Llullaillaco Lake, was established and evaluated.
Material & methods: A genomic DNA library for the Illumina platform was prepared; the quality of the sequences was checked and filtrated using Trimmomatic. The genome was assembled using SPAdes and annotated with the RAST platform.
Results: The *Subtercola* sp. strain DB165 draft genome sequence contained a total of 4.03 Mb, distributed in 153 large contigs with an average GC content of 65.11%, containing 3720 predicted genes of which 3666 are protein-coding and 54 RNAs genes (48 tRNA, 6 rRNA). 1448 (39%) encoding genes had a putative function whereas 2272 (61%) annotated as hypothetical proteins. The genome contains an extensive battery of genes to cope with the harsh conditions in Llullaillaco Lake: these include mechanisms involved in ROS defense systems such as production of carotenoids, glutaredoxins, superoxide dismutase, and peroxidase. In order to adapt to growth at low temperatures, *Subtercola* encodes genes for the production of compatible solutes and cryoprotectants such as betaine, glycine, and trehalose. Genes involved in the biosynthesis of unsaturated fatty acids make possible the adjustment of membrane fluidity to the low temperatures. Cold shock proteins involved in the transcription of genes at low temperature were also found. The bacterium can further accumulate polyhydroxybutyrate and polyphosphates to survive starvation conditions The strain has a powerful DNA repair system, which consists of 51 genes, comprised of nucleotide excision repair, photolyase, homologous recombination, non-homologous end-joining ligases, nucleotide base excision repair, as well as nucleotide mismatch excision repair.
Conclusion: The findings indicate that *Subtercola* sp. strain DB165 has a number of genes involved in adaptation to the cold environment such as the production of compounds and enzymes to cope ROS. The genome of *Subtercola* sp. DB165 gives insight into the adaptation and survival strategies of the new isolate which might be indicative also of survival mechanisms employed by other Actinobacteria.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

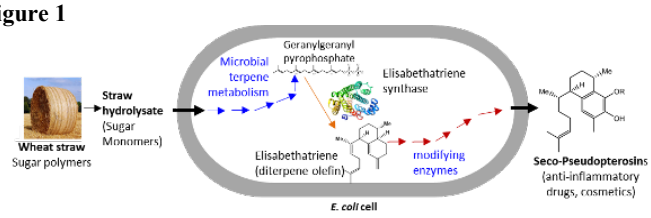
499/GMGP

Production of coral derived Seco-Pseudopterosin derivatives in an engineered *E. coli* strain

M. Reinbold*[1], T. Brück[1]
[1]*Technische Universität München, Chemistry, Garching, Germany*

Seco-Pseudopterosins and Pseudopterosins are diterpene glycosides, derived from the Caribbean soft coral *Antillogorgia elisabethae*. Both compounds show anti-inflammatory activity and have a growing market in the cosmetics and pharmaceutical industry. Current production is derived from wild harvested coral, which poses environmental and supply issues for an expanding market. In this project we aim to identify key biosynthetic elements from coral and other organisms using proteomic and genomic based methods. The sequence data is used to construct a heterologous terpenoid production system based on *E. coli*. Wheat straw hydrolysate will be used as a sustainable carbon source. To regulate metabolic flux, the biosynthetic elements will be integrated into the bacterial genome.

Figure 1



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

500/GMGP

A *Clostridioides difficile* bacteriophage genome encodes binary toxin-associated genes

T. Riedel*[1], J. Wittmann[1], B. Bunk[1,2], I. Schober[1], C. Spröer[1,2], B. Abt[1,2], S. Gronow[1,2], J. Overmann[1,2]
[1]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[2]*German Center for Infection Research (DZIF), Partner site Hannover-Braunschweig, Braunschweig, Germany*

Introduction: Spore-forming pathogenic clostridia typically produce toxins as virulence factors which can cause severe diseases of both humans and animals. These toxins are often encoded on the chromosome, but in some cases they were also found to be encoded on plasmids, e.g., in *Clostridium perfringens*, *Clostridium botulinum*, *Clostridium tetani* or *Clostridium sordellii*. In contrast, toxin-encoding extrachromosomal elements have so far not been detected in *Clostridioides difficile*. During analysis of the complete genome of strain *C. difficile* Semix9, we detected two extrachromosomal elements revealing similarities to bacteriophages, one of them was found to encode binary toxin-associated genes.
Materials and methods: Complete genome sequencing was carried out on the PacBio *RSII*. Genome assembly was performed with the RS_HGAP_Assembly.3 protocol (SMRT Portal) and yielded the complete genome of bacteriophage phiSemix9P1. A final genome quality score of QV60 was attained during resequencing using the RS_Bridgemapper.1 protocol (SMRT Portal). Automated genome annotation was performed using Prokka, followed by manual curation. For expression analysis, extracted and purified RNA was reverse transcribed into cDNA. Genes of interest were checked for expression in induced and non-induced samples.
Results: The bacteriophage phiSemix9P1 has a genome size of 56,606 bp, with a coding percentage of 82.2% and a G+C content of 26.69%. To our knowledge, this bacteriophage genome represents the first temperate *C. difficile* bacteriophage genome harboring and encoding a complete functional binary toxin locus. BLASTN and phylogenetic analysis of the phiSemix9P1 genome revealed only weak similarities to other genetic elements and bacteriophages of *C. difficile*. Annotation and ORF prediction resulted in 74 coding sequences, 36 of them contained conserved domains at the amino acid sequence level. Generally, the bacteriophage genome could be divided into functional clusters that encode DNA packaging, head and tail morphogenesis or host cell lysis. Further, genome analysis of the bacteriophage phiSemix9P1 revealed the presence of genes similar to *cdtR* (response regulator), *cdtA* (ADP-ribosyltransferase subunit CdtA) and *cdtB* (ADP-ribosyltransferase binding protein CdtB) which are representing the binary toxin locus (CdtLoc) of *C. difficile*.
Conclusion: The mobility of virulence factors such as toxins is of interest with respect to the study of the origin and spread of pathogenic strains. The genome of phiSemix9P1 shows for the first time a complete mobile binary toxin locus in a bacteriophage genome which likely is relevant for the transfer of the toxin to other *C. difficile* strains.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

501/GMGP

Resistance phenotype prediction of clinical *Pseudomonas aeruginosa* isolates based on molecular markers combined with genotype-phenotype correlations via an interactive database (Bactome)

A. Khaledi*[1], M. Schniederjans[1], A. Weimann[1], A. McHardy[1], K. Hornischer[1], S. Häußler[1]
[1]*Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany*

The increasing emergence of antimicrobial resistances in combination with the lack of new antibiotic drugs candidates underscores the need for alternative strategies to combat bacterial infections. One option here is the optimization of diagnostics to enable a faster and more targeted therapy and at the same time diminish the evolution and spread of multidrug resistance. This may be accomplished by the identification of phenotype-related molecular biomarkers, as the antibiotic resistance status of a bacterial pathogen is defined by its genome.
We applied next-generation sequencing (NGS) technologies to record the genomic and transcriptomic data of more than 400 clinical *Pseudomonas aeruginosa* isolates. This information was combined with phenotypic profiles (i.e. antibiotic resistance) to identify single phenotype-associated adaptive variations as well as global patterns of common gene expression changes and sequence mutations. We were able to identify distinct mutation and expression patterns associated with resistance to the antibiotic classes of fluoroquinolones and β-lactams, and use these markers for surprisingly accurate phenotype prediction. Interestingly, many of the identified markers do not seem to be directly associated with known antibiotic resistance mechanisms, but rather appear to be secondary or compensatory alterations.
Furthermore, we developed an interactive web-based database (Bactome) to facilitate storage and accessibility of the generated data. Bactome integrates the genomes, transcriptomes and diverse phenotypes of all clinical isolates and can be used to extract sequence and expression information on any single gene or isolate of interest. Additionally, Bactome allows specific data filtering on groups of phenotypically related clinical isolates to determine significantly differentially expressed genes or frequently mutated positions in these isolates.
Concluding, our research serves the establishment of genotype-based molecular tools to pave the way for faster diagnostics and more efficient, targeted treatment strategies.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

502/GMGP

Complete genome sequence of thermophilic xylan-degrading strain *Thermus brockianus* GE-1

C. Schäfers*[1], S. Blank[1], S. Wiebusch[1], S. Elleuche[1], G. Antranikian[1]
[1]*Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany*

Introduction: *Thermus brockianus* strain GE-1 is a thermophilic, Gram-negative, rod-shaped and non-motile bacterium that was isolated from the Geysir geothermal area, Iceland. Based on its thermophilic nature, this strain has been used in activity-based screening approaches for the identification of novel heat-stable biocatalysts. The strain *T. brockianus* GE-1 was chosen for whole genome sequencing due to its ability to use xylan as sole carbon source. To our knowledge the hydrolysis of xylan has not been described for any other *T. brockianus* strain so far. With the description of the corresponding thermostable xylanase, Xyn10, we already identified and characterized one of the key enzymes in a putative xylan degradation pathway of *T. brockianus* GE-1. Xyn10 exhibits its highest activity at 95 °C and pH 6. It also

shows remarkable high stability in the presence of detergents, representing a promising biocatalyst for industrial process at elevated temperatures [1].
Objectives: To gain a deeper understanding of the polymer degrading pathways, we performed a whole *de novo* genome sequencing approach of *T. brockianus* strain GE-1 by using the third generation sequencing technology platform of Pacific Biosciences (PacBio RS II).
Results: Here, we present the first whole genome sequence of a *T. brockianus* strain with finished grade status, showing a phred quality value of QV50. The complete genome size is 2.38 Mb, comprising the chromosome, the megaplasmid pTB1 and the smaller plasmid pTB2. Gene prediction revealed 2,511 genes in total, including 2,458 protein-encoding genes, 53 RNA and 66 pseudo genes. Further insights of the genome sequence, including an overview of annotated sequences that encode putative biocatalysts will be described in more detail as well as a description of the xylanolytic degradation pathway of *T. brockianus* GE-1.
Conclusions: The description of the xylanolytic degradation pathway and the key enzymes of *Thermus* spp. will contribute in finding novel enzymes that are of interest for industrial application.

References
[1] Blank, S., et al., Biochemical characterization of a recombinant xylanase from *Thermus brockianus*, suitable for biofuel production. JSM Biotechnol. Biomed. Eng, 2014. 2: p. 1027.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

503/GMGP
The cystic fibrosis upper and lower airways microbial metagenome of patients with cystic fibrosis or immune deficiency
K. Pienkowska*[1], P. Moran Losada[1], S. Fischer[1], R. Hyde[1], S. Hedtfeld[1], M. Dorda[1], U. Baumann[1], L. Wiehlmann[1], B. Tümmler[1]
[1]*Medizinische Hochschule Hannover, Hannover, Germany*

Cystic fibrosis (CF) is the most common severe autosomal recessive trait in Germany that is caused by mutations in the *Cystic Fibrosis Transmembrane Conductance Regulator* (*CFTR*) gene. The basic defect of reduced chloride and bicarbonate secretion predisposes to chronic airway infections with opportunistic pathogens. We applied shotgun metagenome sequencing to resolve the complex poly-microbial communities in upper and lower airways by collecting nasal lavage, throat swabs and induced sputa from 41 exocrine pancreatic insufficient (PI) CF patients, 21 exocrine pancreatic sufficient (PS) CF patients and 10 patients with immune deficiency (ID, positive disease control).
Results. The samples contained on the average 97% of human DNA. In the remaining 3% of DNA hundreds of bacterial taxa and less than a dozen fungi, molds or DNA viruses were detected. Most viruses were bacteriophages accompanied by adeno- and herpesviruses in a few samples. Least bacterial species diversity was seen in the lungs of PI CF patients. The microbial communities were dominated by the opportunistic pathogens known from culture-dependent diagnostics, i.e. *Staphylococcus aureus, Pseudomonas aeruginosa, Haemophilus influenzae, Stenotrophomonas maltophilia* plus some Rothia, Veilonella and Prevotella spp. The metagenomes became more and more similar with the progression of lung disease severity. In contrast, most bacterial species diversity was seen in the most mildly affected group of PS CF patients who presented metagenomes like those of healthy controls dominated by Streptococcus, Veilonella and Rothia spp. Nevertheless the microbial communities in nose, throat and lung were more similar between PI and PS CF than with ID. Moraxella and Haemophilus spp. were abundant in ID patients, but only minor members of the CF airway communities. In CF sputa the presence of Streptococci and Neisseria and of Bifidobacteria, Veilonella, Prevotella, Atopobium, Fusobacterium and the emerging pathogen *Streptococcus anginosus* was positively correlated with each other. In summary, we observed both habitat- and disease-specific signatures in the three niches and the three disease cohorts.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

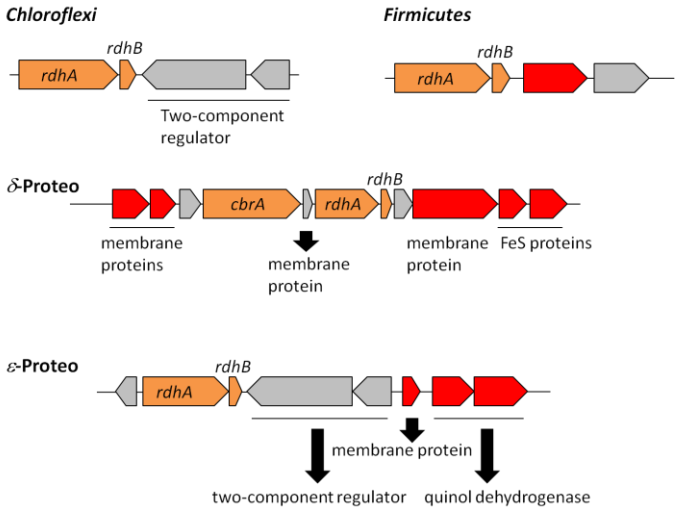
504/GMGP
Comparative Genomics of Organohalide-Respiring Bacteria Suggest the Presence of Four Different Organohalide-Respiratory Chains
T. Goris*[1]
[1]*Friedrich Schiller Universität Jena, Angewandte und Ökologische Mikrobiologie, Jena, Germany*

Organohalide respiration is a special type of anaerobic respiration, in which halogenated organic compounds are used as electron acceptor. The terminal reductases in this process are reductive dehalogenases - hitherto the only known corrinoid-dependent periplasmic enzymes and terminal reductases. Further components of the organohalide-respiratory chains are uncharacterized. The bacteria carrying out this environmentally important respiration belong to the Firmicutes (*Desulfitobacterium*, *Dehalobacter*), Chloroflexi (*Dehalococcoidia*), δ- and ε-Proteobacteria (*Geobacter*, *Anaeromyxobacter*, *Desulfomonile*; *Sulfurospirillum*). During the last five years, many genomes of organohalide-respiring bacteria (OHRB) were sequenced and analyzed, leading to a wealth of genomic information available about this phylogenetically very diverse group. When comparing the RDase-encoding gene clusters, highly diverse accessory proteins are found to be encoded, differing enormously when comparing the RDase gene regions. The number of genes putatively involved in the whole process of organohalide respiration range from four to more than 30 and often include genes encoding membrane and/or iron-sulfur cluster-containing proteins. Here, the comparative analysis of OHRB genomes from all four phyla is presented and hypotheses for the architecture of the corresponding organohalide respiratory apparatus are developed. Each phylum seems to have its own distinct respiratory chain architecture: ε-Proteobacteria seem to employ a special type of quinol dehydrogenase (Goris et al., 2014 and 2015). Firmicutes encode a putative flavin-containing membrane protein. Dehalococcoidia might form an unusual respiratory chain not incorporating any quinones, as was recently reported (Kublik et al., 2015). δ-Proteobacteria have the most diverse OHR gene clusters. One resembles that of ε-Proteobacteria, another the Firmicutes-type, while the third one is unique: It encodes several ferredoxin-like proteins as well as membrane proteins and two RDases. The involvement of these proteins in OHR is underpinned by first transcription studies. Also discussed are differences regarding the accessory proteins for organohalide respiration, e.g. proteins involved in RDase maturation or biosynthesis of the corrinoid cofactor.
The outcome of this comparative genomics approach underlines the diversity and uniqueness of this type of anaerobic respiration as well as the necessity to biochemically characterize the organohalide respiration machinery in all four phyla of OHRB.

References
[1] Goris T, et al. (2014), Environ Microbiol. Nov;16(11):3562-80.
[2] Goris T, et al. (2015), Sci Rep. Sep 21;5:13794.
[3] Kublik A, et al. (2016), Environ Microbiol. Sep;18(9):3044-56.

Figure 1: Archetypical reductive dehalogenase gene clusters of four different phyla. *rdhA*: reductive dehalogenase catalytic subunit, *rdhB*: reductive dehalogenase membrane anchor, orange: RDase genes, red: genes encoding putative electron transfer proteins

Figure 1



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

505/GMGP

Dynamics of *Mycobacterium tuberculosis* pyrazinamide resistance: Effect of lineage specific mutations

R. Baddam*[1], N. Kumar[2], L. H. Wieler[3], A. K. Lankapalli[4,5], N. Ahmed[5], T. Semmler[1]
[1]*Robert Koch Institut, NG1, Berlin, Germany*
[2]*University of Cambridge, Cambridge, United Kingdom*
[3]*Robert Koch Institut, Berlin, Germany*
[4]*Max Planck Institute for the Science of Human History, Jena, Germany*
[5]*University of Hyderabad, Hyderabad, India*

Pyrazinamide (PZA) is an important first-line drug included in treatment of both susceptible and multidrug resistant *Myobacterium tuberculosis*. Resistance to PZA is primarily due to acquisition of mutations in pncA gene (Rv2043c) which encodes pyrazinamidase enzyme responsible for conversion of pro-drug PZA into its active form. Past studies have observed that mutations in pncA gene are diverse in nature and scattered across the gene without any clear hotspot regions. As different lineages of *M. tuberculosis* display strong geographic association, we sort to understand the impact genetic background may have on acquisition of PZA resistance. For this, we analyzed sequence data of 1480 clinical isolates representing all four major lineages. As pncA gene is located in an operon comprising of two other neighboring genes, we identified genetic variants in the complete operon and its upstream promoter region. The analysis led to identification of a lineage specific frame-shift mutation in Rv2044c gene located upstream of pncA, which disrupted the stop codon leading to its fusion with pncA gene. This in turn added a novel domain of unknown function DUF2784 to original pyrazinamidase enzyme encoded by pncA gene. The variant pyrazinamidase molecule was also computationally modelled and various physico-chemical parameters were determined and compared. The lineage specific nature of this mutation and its potential alteration in one of the key gene pncA highlights the importance of studying lineage specific differences that might underpin the variable acquisition of drug resistance in different geographical settings.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

506/GMGP

Comparative genomics of the global gastric diversity of *Helicobacter pylori*

F. Ailloud*[1], S. Woltemate[2], F. Kops[2], B. Brenneke[2], X. Didelot[3], B. Bunk[4], C. Spröer[4], J. Overmann[4], C. Schulz[5], P. Malfertheiner[5], S. Suerbaum[1]
[1]*Max von Pettenkofer-Institut, Munich, Germany*
[2]*Medizinische Hochschule Hannover, Hannover, Germany*
[3]*Imperial College London, London, United Kingdom*
[4]*Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures , Braunschweig, Germany*
[5]*Otto-von-Guericke-Universität, Universitätsklinik für Gastroenterologie, Hepatologie und Infektiologie, Magdeburg, Germany*

Helicobacter pylori is a pathogenic bacteria restricted to the human stomach. The infection is commonly acquired during childhood and can successfully establish life-lasting chronic colonization if not treated. This organism is characterized by an unusually high genetic diversity resulting from increased recombination and mutation rates. Since it is restricted to a single ecological niche, mixed infections are the only opportunities for *H. pylori* to acquire extraneous genetic material via recombination or horizontal gene transfer. Stomach environmental conditions are constantly evolving due to mucosa regeneration, diet, antibiotic consumption and in response to the infection, thus challenging the bacteria with variable levels of inflammation and acid secretion.
H.pylori genome-wide diversity has been studied mostly using few isolates sampled in closely-related individuals or at different time points in a single one. In order to characterize the genetic variation and population dynamics associated with the colonization of the whole human stomach, gastric biopsies from the antrum, corpus and, in some cases, fundus were obtained from 7 *H. pylori* infected individuals and cultured. 10 to 15 *H. pylori* clones were isolated from each biopsy, and their genomes sequenced with an Illumina MiSeq instrument. In addition, the genomes of one to three isolates per patient were analyzed using Single Molecule, Real-Time (SMRT) sequencing technology to obtain closed reference genomes and methylome data.
Within-host diversity was characterized with different bioinformatics tools. All genomes were found to belong to the hpEurope population and no mixed infections with multiple unrelated strains were detected. A wide range of recombination to mutation ratios (r/m) was observed between different infected humans. Within the same patient, comparative analyses often revealed large genome rearrangements, differential methylation and variation in the distribution of virulence factors such as the cag pathogenicity island or outer membrane proteins. Isolates sampled from the same biopsy did not always cluster together in whole-genome phylogenies, suggesting a frequent mixture of populations between gastric regions. Using a molecular clock rate of 1.9e-5 per year per site, the time to the most recent common ancestor (TMRCA) between isolates from a single stomach were estimated and ranged from 5.4 months to 7 years. No correlation could be established between TMRCA and patients ages. Altogether, these results highlight the necessity to consider the global gastric diversity of *H. pylori* populations in order to fully understand the dynamics of colonization, and suggest that major evolutionary events might periodically reshape within-host diversity.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Gene Regulation and Non-coding RNA (FG GR)

507/GRP
The involvement of the antisense RNA RSaspufL in regulated formation of photosynthesis complexes in *Rhodobacter sphaeroides*
C. Reuscher*[1], G. Klug[1]
[1]*Institute for Micro- and Molecularbiology, Giessen, Germany*

The facultative phototrophic model bacterium *Rhodobacter sphaeroides* is known for its metabolic versatility. Under low oxygen conditions it synthesizes intracytoplasmic membranes harboring 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 for the light harvesting complex I (LHI) and of the reaction centre (RC).
The *pufL* sequence is quite conserved among different *Rhodobacter* species (78% identity between *R. sphaeroides* 2.4.1 and *R. capsulatus* SB1003). Nevertheless *Rhodobacter capsulatus pufL* contains an RNase E cleavage site which is not conserved in *R. sphaeroides* at the same position. This RNase E cleavage site is essential for differential *puf* mRNA processing and degradation which contributes to the stoichiometry of LHI and RC complexes in *R. capsulatus*. Comparative RNAseq from a mutant which harbors a thermosensitive RNAse E variant instead of the wildtype RNase E and the wildtype *R. sphaeroides* 2.4.1 revealed an RNAse E cleavage site in the *pufL* region in *R. sphaeroides* 110 bp upstream of the cleavage site of *R. capsulatus*.
RNAseq and *Northern blot* analysis of transcripts derived from the *puf* operon unveiled that also certain small RNAs (sRNAs) are transcribed. Up to date two different *puf* operon associated sRNAs (RSspufX and RSaspufL) were identified. One abundant putative sRNA, RSspufX, was detected downstream of *pufX* cotranscribed with the *puf* operon (Berghoff et al., 2009). Another, less abundant sRNA RSaspufL was detected antisense to the 5´region of the *pufL* gene extending into the *pufA-pufL* intercistronic region and possibly even further. Northern blot results confirmed the presence of RSaspufL (about 200 nucleotides) under microaerobic and phototrophic conditions. Similar to the expression of the *puf* operon the transcription of RSaspufL depends on the response regulator PrrA.
An artificial increase in the amount of the RSaspufL by plasmid driven over-expression led to an alteration in the absorption spectra which is characteristic for the LHCI und the RC. Because of the fact that RSaspufL overstretches the RNAse E and the Shine-Dalgarno sequence of *pufL* we hypothesize that the antisense RNA RSaspufL contributes to the regulated processing and degradation of the *puf* mRNA in *R. sphaeroides*. The influence of RSapufL on the *puf* mRNA should be investigated via Northern blot analysis, Real-Time PCR and reporter gene fusion. We will present data on the effect of RSaspufL on *pufL* mRNA levels and stabilities.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

508/GRP
Differential regulation of Nar2 between mycelium and spores in *Streptomyces coelicolor*
D. Falke*[1], M. Fischer*[1], T. Damelang[1], P. Hase[1], G. Sawers[1]
[1]*Martin-Luther-Universität, Institut für Biologie/Mikrobiologie, Halle, Germany*

Introduction: *S. coelicolor* A3(2) is a soil-dwelling microorganism with at least two completely different developmental stages: unicellular spores and filamentous vegetative mycelia. Spores represent a semi-dormant stage with strongly reduced anabolic and catabolic metabolisms. Nevertheless they are able to respire oxygen and to consume extracellular glucose. These reactions ensure maintenance of a membrane potential and re-stock the intracellular trehalose reserves. Interestingly, under anaerobic conditions spores are able to activate an extant nitrate reductase (Nar1) that allows anaerobic respiration with nitrate . Nitrate respiration also takes place in exponential mycelium if it becomes hypoxic or anaerobic, but this is performed by another nitrate reductase called Nar2. The synthesis of the Nar2 enzyme in mycelium is regulated by the two-component system SCO0203 and SCO0204. Due to the fact that expression of the operon encoding Nar2 is independent of nitrate, the sensor kinase detects something other than nitrate. Recently, we could demonstrate that Nar2 synthesis is induced by hypoxia both in mycelium and in resting spores.
Objective: To characterize the influence of the sensor kinase SCO0203 on the synthesis and activity of Nar2 in mycelium and spores of *S. coelicolor* A3(2).
Material & methods: A mutant that is defective in SCO0203 was characterized by western blot analyses for the presence of the catalytic subunit of Nar2 (NarG2). These analyses were implemented with crude extracts of hypoxically incubated spores, as well as with vegetative mycelium and were complemented by introducing a wild-type copy of the gene *SCO0203* into the genome of the affected mutant.
Results: A mutant with a transposon-insertion in *SCO0203* fails to synthesize several gene products encoded at the locus adjacent to *SCO0203*. It was shown that the previously characterized nitrate transporter NarK2 (SCO0213), a putative hemerythrin (SCO0212) and the Nar2 enzyme (SCO0216-SCO0219) could not be detected in crude extracts of exponentially growing mycelium derived from the *SCO0203* mutant using specific antibodies. Hypoxic incubation of resting spores revealed that synthesis of Nar2 could be induced; induction did not occur after either aerobic or anaerobic induction. In contrast to mycelium, a defective *SCO0203* gene did not prevent hypoxic induction of Nar2 synthesis in spores.
Conclusion: The *SCO0203* gene product is a histidine kinase essential for the initiation of Nar2 synthesis in mycelium but it is not required for the hypoxic induction of Nar2 synthesis in resting spores. The existence of another regulation pathway sensing hypoxia in spores must be assumed.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

509/GRP
RNase E and RNase J are needed for S-adenosylmethionine homeostasis in Sinorhizobium meliloti
H. Melior*[1]
[1]*Molekularbiologie & Mikrobiologie JLU Gießen, AG Evguenieva-Hackenberg, Gießen, Germany*

The ribonucleases (RNases) E and J are essential in *Escherichia coli* and *Bacillus subtilis*, respectively, and co-exist in *Sinorhizobium meliloti*. We analyzed *S. meliloti* 2011 mutants with mini-Tn5 insertions in the corresponding genes rne and rnj and found many overlapping effects. We observed similar changes in mRNA levels including lower mRNA levels of the motility and

chemotaxis related genes flaA, flgB, and cheR and higher levels of ndvA (important for glucan export). The acyl-homoserine lactone (AHL) levels were also higher during exponential growth in both RNase mutants, despite no increase in the expression of the sinI AHL synthase gene. Furthermore, several RNAs from both mutants migrated aberrantly in denaturing gels at 300 V but not under stronger denaturing conditions at 1300 V. The similarities between the two mutants could be explained by increased levels of the key methyl donor S-adenosylmethionine (SAM), since this may result in faster AHL synthesis leading to higher AHL accumulation as well as in uncontrolled methylation of macromolecules including RNA, which may strengthen RNA secondary structures.
Indeed, we found that in both mutants the SAM level was increased at least sevenfold. Complementation by induced ectopic expression of the respective RNase restored the AHL and SAM levels in each of the mutants. In summary, our data show that both RNase E and RNase J are needed for SAM homeostasis in *S. meliloti*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

510/GRP
The 1232 nt long non-coding RNA SSR42 regulates virulence in *Staphylococcus aureus* on a transcriptional level
J. Horn*[1], M. Klepsch[1], B. Huettel[2], R. Reinhardt[2], T. Rudel[1], M. Fraunholz[1]
[1]*University of Wuerzburg, Chair of Microbiology, Wuerzburg, Germany*
[2]*Max Planck Genome Center, Cologne, Germany*

Stapyhlococcus aureus is a successful human pathogen causing a multitude of diseases by a plethora of virulence factors. In a previously conducted transposon screen we identified *repressor of surface proteins* (Rsp) as a novel virulence regulator [1]. Rsp directly controls the 1232 nt long non-coding RNA SSR42 (also known as RsaX28, teg27).
Here we show that SSR42 is essential for hemolysis by regulating transcription of the pore-forming α-hemolysin during the stationary growth phase of *S. aureus*. By mutation we identified functional domains within the ncRNA and determined the transcriptome of a SSR42 mutant by RNAseq. Our data revealed that SSR42 is the main effector for Rsp-mediated virulence in *S. aureus* by controlling the transcription of a variety of staphylococcal virulence factors. We hence generated a reporter plasmid to assess activation of the SSR42 promotor. Promotor activity was detected after exposure to various antibiotics and stresses illustrating enhanced virulence of *S. aureus* upon treatment with subinhibitory concentrations of antibiotics.
References
[1] Das S., Lindeman C. et al. (2016) Natural mutations in a Staphylococcus aureus virulence regulator attenuate cytotoxicity but permit bacteremia and abscess formation. PNAS Vol. 113 No. 22, E3101-E3110.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

511/GRP
A high-resolution genome annotation of *Staphylococcus aureus* HG003 and functional analysis of newly detected sRNAs
S. H. Yu*[1], P. Tanwer[2], M. Sharan[2], M. Sauer[2], C. Schuster[3,4], A. Smirnov[2], A. Herbig[5], R. Bertram[3,6], K. Nieselt[5], K. Förstner[2,1], J. Vogel[2]
[1]*University of Würzburg, Core Unit Systems Medicine, Würzburg, Germany*
[2]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*
[3]*University of Tübingen, Department of Microbial Genetics, Tübingen, Germany*
[4]*Imperial College London, London, United Kingdom*
[5]*University of Tübingen, Center for Bioinformatics Tübingen, Tübingen, Germany*
[6]*Paracelsus Medical University, Research Department, Nuremberg, Germany*

Due to its sensitivity to all known antibiotics *Staphylococcus aureus* NCTC8325 is widely used for studying antibiotic resistance transferred by plasmids. *Staphylococcus aureus* HG003 is a derivative of NCTC8325 in which the *rsbU* (activator of SigB encoding sigma factor B) and *tcaR* (activator of protein A transcription) are repaired. This strain shows weak hemolytic acitivity, high *spa* transcription levels, strong biofilm formation and high virulence. This makes it a potent model to study the regulation of virulence. So far there is no comprehensive genome annotation of *S. aureus* HG003 available. Using RNA-Seq of 14 conditions and computational predictions we have globally identified numerous important transcriptomic features including 2588 transcripts, 267 small RNAs (sRNA) of which several are UTR-derived, 17 riboswitches and 143 potential small open reading frames. Based on the expression profiles we allocated the sRNAs to co-regulated mRNAs and derived potential function by this association. Our high-resolution genome annotation is a rich resource for the *S. aureus* community. The most promising sRNAs are going to be further examined experimentally.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

512/GRP
Complex transcriptional regulation of the *isc-suf*-operon in *Rhodobacter sphaeroides* – How complex is complex?
K. Werler*[1], B. Remes[1], G. Klug[1]
[1]*Justus-Liebig-University, Institute for micro- and molecular biology, Giessen, Germany*

Question: In the facultative phototrophic α-proteobacterium *Rhodobacter sphaeroides* the formation of photosynthetic complexes is enhanced at low oxygen tension and requires Fe-S-cluster assembly, since these clusters are essential components of the photosynthetic complexes. But Fe-S-cluster underlay several destabilizing effects. The most important one are the cytotoxic reactive oxygen species (ROS), which lead to the breakdown of Fe-S-cluster, the release of free iron and thereby the production of cytotoxic hydroxyl radicals. To protect the cellular surrounding from the cytotoxic effect of free iron, the formation of Fe-S-cluster needs to be well controlled. Up to date the *isc* (iron-sulfur cluster) and *suf* genes are known to encode the most prominent Fe-S-biogenesis systems. In *E.coli* these genes are organized in two different operons to allow a transcriptional switch upon changing environmental conditions. In *Rhodobacter sphaeroides* the *isc* and *suf* genes are organized in one operon and the transcriptional control of the gene expression is expected to be very complex.
Methods & Results: First of all there is evidence for individual promoters for *isc* and *suf* genes, yet also for co-transcription. Differential RNAseq analyses performed by [1] revealed multiple mRNA transcripts, which are sense and antisense to the operon and hints towards several promoter regions. Additionally the complex regulation of the genes via multiple sense- and antisense

promoters seems to be influenced by iron availability and oxygen concentration. On top of that the operon is likely to be affected by different protein regulators like IscR, Irr, Fur/Mur, OxyR, PrrA or RirA-like homologs acting as transcription factors. To further elucidate the predicted promoters of the operon *in vivo* we fused single and multiple promoters to a *lacZ*-based reporter system. Surprisingly a promoter for transcription in antisense direction showed significantly higher activity than several promoters transcribing in sense direction to *iscRS*. To further elucidate the impact of predicted regulators of the *isc-suf* operon and to narrow down the binding sites of the different regulators the reporter plasmids were transferred to several mutant strains lacking negative and positive regulators of the *isc-suf* operon. Interestingly the expression of the reporter system in an OxyR-mutant strain revealed an increased activity of antisense promoter 5, indicating a possible binding site of the protein regulator in front of *iscR*.
Conclusions: We will present data about the growth behavior of the mutant strains and from *in vivo* reporter assays so that the complex regulation of the *isc-suf* operon can be stepwise unraveled.

References
[1] Remes, B., Eisenhardt, B. D., Srinivasan, V., & Klug, G. (2015). IscR of *Rhodobacter sphaeroides* functions as repressor of genes for iron-sulfur metabolism and represents a new type of iron-sulfur-binding protein. MicrobiologyOpen, 4(5), 790-802.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

513/GRP
Translational coupling via termination/reinitiation in *Haloferax volcanii*
S. Laaß*[1], E. Kolbe[1], C. Wehrheim[1], J. Soppa[1]
[1]*Institute for Molecular Biosciences, Goethe-University, Frankfurt, AK Soppa, Frankfurt am Main, Germany*

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 based on the sequestration of the Shine-Dalgarno (SD) motif of the downstream gene in a secondary structure and unmasking of the SD motif by a ribosome translating the upstream gene. Another mechanism of translational coupling termed termination-reinitiation is known from eukaryotic viruses and can operate on closely spaced or overlapping genes. In this case ribosomes that terminate translation of an upstream gene remain on the transcript (at least the small subunit) and reinitiate translation on a downstream gene.
The genome of the haloarchaeon *Haloferax volcanii* contains 4128 genes, 886 of which form 443 overlapping gene pairs1. These gene pairs typically have 4 nucleotide (A**TG**A) or 1 nucleotide (TG/A**A**TG) overlaps. It was observed that in *H. volcanii* SD motifs in 5`UTRs are not involved in translation initiation. However, SD motifs are enriched in the upstream genes with an optimal distance to the start codon of the downstream genes, and it has been postulated that they might be involved in termination-reinitiation1. To test experimentally whether termination-reinitiation operates in *H. volcanii* nine gene pairs were selected and the upstream genes were fused with the native overlap to the reporter gene *dhfr* (dihydrofolate reductase). In each case variants were generated that contained a stop codon in the upstream gene, inhibiting ribosomes from reaching the overlap. The levels of the bicistronic transcripts as well as the DHFR levels were quantified. Strikingly, the DHFR levels were zero when ribosomes terminated prematurely, showing that termination-reinitiation operates in *H. volcanii* and there is no *de novo* initiation at the downstream gene. Total replacement of the SD motif in two proof-of-principle examples resulted in a drastic decrease in the DHFR level, indicating that the SD motif is essential for efficient termination-reinitiation. Further analyses of the importance of the SD motif for the efficiency of termination-reinitiation are currently under way.
Possible evolutionary advantages of termination-reinitiation (compared to independent initiation) will be discussed.

References
[1] Kramer et al., (2014) *Haloferax volcanii*, a species that does not use the SD mechanism for translation initiation at 5-UTRs. PLoS ONE 9,394979.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

514/GRP
Translational coupling via termination/reinitiation in *Escherichia coli*
M. Huber*[1], K. Vogtmann[1], S. Laaß[1], J. Soppa[1]
[1]*Institute for Molecular Biosciences, Goethe University, Frankfurt am Main, Germany*

Many genes in bacteria are organized in operons and are transcribed into polycistronic mRNAs. This enables translational coupling, meaning translation of a downstream gene depends on translation of an upstream gene. One mechanism of translational coupling is based on the sequestration of the Shine-Dalgarno (SD) motif of the downstream gene in a secondary structure and unmasking of the SD motif by a ribosome translating the upstream gene. Another mechanism of translational coupling termed termination-reinitiation is known from eukaryotic viruses and can operate on closely spaced or overlapping genes. In this case ribosomes that terminate translation of an upstream gene remain on the transcript (at least the small subunit) and reinitiate translation on a downstream gene.
Bacteria have many gene pairs that overlap by 4 nt (A**TG**A) or by 1 nt (TG/A**A**TG). The genome of the bacteria *Escherichia coli* contains 4288 genes, 820 of which form 410 overlapping gene pairs. Five gene pairs were selected, which overlapped by 4 nt to test experimentally whether termination-reinitiation operates in *E. coli.* A dual reporter gene system was established and translational fusions were constructed of 1) the reporter gene *glpD* and the last 33 codons of the selected upstream genes, and 2) the first ten codons of downstream genes and the reporter gene *gusA*. In each case variants were generated that contained a stop codon at the end of the *glpD* gene, inhibiting ribosomes from reaching the gene overlap. The levels of the bicistronic transcripts as well as the GlpD and GusA levels were quantified. In four of the five cases the GusA activity could only be detected when the ribosomes reached the gene overlap and it was zero in the version with the stop codon. These results showed that termination-reinitiation operates in *E. coli, de novo* initiation at the downstream gene occurred only at one of five gene pairs. To investigate the role of the SD motif in termination-reinitiation, it was mutated via site-directed mutagenesis for two gene pairs. The reduction of the number of base pairs between transcript and 16S rRNA led to a considerable reduction of the GusA activity, and the GusA activity was close to zero after total replacement of the SD motif. These results underscore that the SD motif is essential for termination-initiation to occur, most probably because it is needed to keep the ribosome attached to the transcript after termination of translation of the upstream gene. Possible evolutionary advantages of termination-reinitiation (compared to independent initiation) will be discussed.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

515/GRP

Systems-level modelling of the bacitracin resistance network in *Bacillus subtilis* rationalizes interdependence between resistance modules

H. Piepenbreier*[1], G. Fritz[1]
[1]*LOEWE Zentrum für Synthetische Mikrobiologie, Marburg, Germany*

In the fierce competition for limited resources, many organisms secrete antimicrobial peptides (AMPs) that inhibit the growth of competing species. In response to such attacks, bacteria frequently express a whole battery of resistance genes. In *Bacillus subtilis* for instance, resistance against the lipid II cycle-inhibiting AMP bacitracin is mediated via three different resistance modules: The ABC transporter BceAB, the UPP phosphatase BcrC and the phage-shock proteins LiaIH [1]. While all of these modules are well characterized individually, surprisingly little is known about how these resistance modules interact. Only recently, we revealed a clear-cut hierarchy in their ability to protect the cell and found significant redundancy between these modules [2], but a quantitative framework that could rationalize these data was missing. Here, we report on our on-going efforts in closing this gap, which relies on systems-level modelling of the bacitracin resistance network. Our overall model encompasses computational descriptions of the individual resistance modules, which interact with each other by differentially affecting and responding to the pool levels of lipid II cycle intermediates. Strikingly, our preliminary results predict that BceAB and BcrC contribute additively to bacitracin resistance, although our experiments in deletion mutants revealed a significant redundancy between them. However, within our model redundancy emerges as a result of the dynamic regulation within the whole network, which features compensatory up-regulation of secondary resistance modules, if primary resistance fails. In summary, we believe that our systems-level model of a complex AMP resistance network not only provides a powerful tool to decipher the interactions between individual resistance modules, but will also be instrumental to predict the antimicrobial potency of various drug-drug combinations.

References

[1] Radeck J, Fritz G, Mascher T. The cell envelope stress response of *Bacillus subtilis*: from static signaling devices to dynamic regulatory network. Current Genetics. 2016, 1–12. doi:10.1007/s00294-016-0624-0
[2] Radeck J, Gebhard S, Orchard PS, Kirchner M, Bauer S, Mascher T, and Fritz G. Anatomy of the bacitracin resistance network in *Bacillus subtilis*. Mol Microbiol. 2016;100: 607–620. doi:10.1111/mmi.13336

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

516/GRP

Transcriptional activity of rDNA promoters in *Corynebacterium glutamicum*

T. Hahn*[1], J. Schöferle[1], S. Weinmann[1], M. Ruwe[2], B. Eikmanns[1], J. Kalinowski[2]
[1]*Unversität Ulm, Mikrobiologie & Biotechnologie, Ulm, Germany*
[2]*Universität Bielefeld, CeBiTec, Bielefeld, Germany*

Introduction: *Corynebacterium glutamicum* is mainly used for the biotechnological production of amino acids. It is a robust producer strain, easy to handle, and suitable for genetic engineering. However, its growth rate is significantly lower than that of other production organisms [1] and thus, there is a need for growth improvement. Possible factors influencing the growth rate are the number and expression of ribosomal DNA *(rrn)* operons encoding the 5S, 16S, and 23S rRNAs.
Objectives: In *C. glutamicum,* there are six *rrn* operons, which are distributed on the genome in the order *rrnA*, *rrnB*, *rrnF*, *rrnD*, *rrnE,* and *rrnC* [2]. In this study, we analyze the transcriptional activity of each of the *rrn* promoters in *C. glutamicum* under different conditions.
Materials & methods: For investigation of the *rrn* transcriptional activity we used the plasmid pET2, containing the promoterless chloramphenicol acetyltransferase (CAT) gene as a reporter gene [3]. The six *rrn* promoter regions were amplified from genomic DNA of *C. glutamicum* and cloned into plasmid pET2. The resulting vectors were transformed into *C. glutamicum* and into single and multiple *rrn* deletion mutants and growth experiments were performed. In the exponential growth phase, the cultures were harvested and after cell lysis, CAT assays were performed to determine specific activities. To eliminate copy number effects of the vector used, we generated integration mutants via homologous recombination using the integrative vector pRIM2 with promoter regions of *rrnF* and *rrnE*. This integration vector harbors also the reporter gene *cat* and allows determination in a single copy system [3].
Results: The promoter activities of the six different *rrn* operons differed significantly from each other, the *rrnF* promoter showing the highest specific activity (up to 10 U mg protein-1) on all media tested. The total specific activity of all six *rrn* promoters was shown to vary on different media. Surprisingly, the *rrnF* and the *rrnE* promoter activities increased and decreased, respectively, with the number of deleted *rrn* operons, indicating a *rrn* regulatory effect. In case of the single copy system pRIM2, we just observed increased promoter activities for *rrnF* and *rrnE* with rising number of *rrn* deletions.
Conclusion: The results indicate that (i) the six *rrn* operons in *C. glutamicum* are differentially expressed, (ii) expression is different on different media, and (iii) the increasing promoter activities of *rrnF* and *rrnE* with the number of deleted *rrn* operons correlate to in vivo *rrn* activity of *C. glutamicum*.
Acknowledgement: This work was supported by the BMBF grant 031A302E.

References

[1] Uthan et al. 2013. Biotechnol. Bioeng. 111: 359-371.
[2] Martin et al. 2003. J. Biotechnol. 104**:** 41-53.
[3] Vasicova et al. 1998. Biotechnol. Tech. 12: 743-746.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

517/GRP

A RisA-dependent small regulatory RNA is involved in glutamate uptake in *Bordetella pertussis*

K. Keidel[1], F. Amman[2], I. Bibova[1], J. Capek[1], A. Jain[1], D. Hot[3], B. Vecerek*[1]
[1]*Institute of Microbiology v.v.i., Prague, Czech Republic*
[2]*Institute for Theoretical Chemistry, Vienna, Austria*
[3] *Institut Pasteur de Lille, Laboratoire Transcriptomique et Génomique Appliquée,, Lille, France*

Question: *Bordetella pertussis* is the causative agent of human whooping cough (pertussis), a highly contagious respiratory disease. We have shown that the RNA chaperone Hfq plays a significant role in physiological fitness of *Bordetella pertussis*. This suggested that Hfq-dependent small regulatory RNAs could be involved in modulation of gene expression in this re-emerging pathogen.
Methods: High through-put RNA sequencing methods combined with biocomputational tools were applied in order to search for sRNAs in *B. pertussis*. Expression of s2013 sRNA and its regulatory functions were analyzed by Northern blot, Western blot and microarray techniques.

Results: Using *in silico* and RNA-seq analyses hundreds of putative sRNAs were identified. One of them, s2013 sRNA was discovered by transcriptomic profiling of *B. pertussis* wt and Δ*hfq* strains using DE RNA-seq methodology. The levels of s2013 were strongly reduced in *hfq* mutant indicating that Hfq affects the stability of the transcript. Interestingly, expression of s2013 was downregulated by activity of the two-component regulatory system BvgAS and, consequently, s2013 levels were elevated in absence of *bvg* genes or under conditions known to silence BvgAS activity. Moreover, the transcription of s2013 sRNA appeared to be dependent on another response regulator, RisA, as the deletion of the *risA* gene completely abolished s2013 sRNA expression. In agreement, several putative RisA binding motifs were identified upstream of the s2013 promoter. Deletion of these motifs obliterated s2013 expression and further confirmed RisA requirement. Profiling of the Δs2013 mutant in Δ*bvgA* genetic background identified BP3831 gene as an exclusive significantly modulated target. BP3831 encodes an ABC periplasmic binding protein with unknown substrate specificity and its transcript levels were increased in absence of s2013. This finding was corroborated also on protein level as BP3831 amounts were elevated in double Δs2013Δ*bvgA* mutant when compared to single Δ*bvgA* mutant. Cultivation of Δs2013 and ΔBP3831 mutants in chemically-defined growth media supplemented with different sources of nutrients revealed that BP3831 is involved in transport of glutamate. *In silico* analysis suggested that s2013 may interact with the ribosome binding site of BP3831 mRNA which plausibly explained the observed negative effect of s2013. In support, mutations resulting in reduction of complementarity between s2013 and BP3831 mRNA led to increased abundancy of BP3831 protein.
Conclusions: Collectively, our data represent the first report on small regulatory sRNA playing a specific role in *B. pertussis* physiology. Expression of small RNA s2013 is subjected to a complex transcriptional control executed by RisA and BvgAS regulators. In the absence of *bvg* genes s2013 attenuates the translation of ABC periplasmic binding protein BP3831 involved in glutamate uptake.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

518/GRP

A special role for acetate kinase AckA in the regulation of CiaR activity in the absence of the cognate kinase CiaH

A. Sexauer*[1], R. Brückner[1]
[1]*TU Kaiserslautern, Microbiology, Kaiserslautern, Germany*

The two-component regulatory system CiaRH of *Streptococcus pneumoniae* is implicated in competence regulation, ß-lactam resistance, maintenance of cell integrity, bacteriocin production, host colonization, biofilm formation and virulence. A surface-exposed protease HtrA and five small noncoding csRNAs, all directly controlled by CiaR, are the major mediators of these phenotypes (4). Expression analyses indicated that the CiaR system is highly active under a variety of growth conditions, not showing an on-off switch typical for many other two-component systems. In addition, depending on the growth conditions, CiaR is active in the absence of its cognate kinase CiaH, although phosphorylation of CiaR is required for DNA binding and gene regulation (2). To determine if acetyl phosphate could be the alternative phosphodonor, genes involved in pyruvate metabolism were disrupted to alter cellular levels of acetyl phosphate. In a CiaH-deficient strain devoid of pyruvate oxidase SpxB, phosphotransacetylase Pta, and acetate kinase AckA, very low acetyl phosphate levels were observed, and in paralell, strongly reduced CiaR-mediated gene expression (3). These results clearly indicate that alternative phosphorylation of CiaR is dependend on acetyl phosphate. A surprising synthetic lethality was detected in CiaH-deficient strains producing high levels of acetyl phosphate. The *ackA* gene could not be inactivated. Furthermore, a strain producing half of the acetyl phosphate level of the wild type lacking AckA showed a 13-fold increase in CiaR-dependent promoter activation. In the absence of AckA, CiaR appears to be extremely activated, provided acetyl phosphate is present and the CiaH kinase is absent. It appears therefore, that alternative phosphorylation of CiaR is affected negatively by AckA. In a first step to determine if this negative regulation is direct, the adenylate reconstituion *Escherichia coli* two hybrid system (1) was applied to detect interaction between CiaR and AckA. The results of these experiments clearly demonstrated contact between these two proteins. The surprising link of the response regulator CiaR to a metabolic enzyme, AckA, adds another level of complexity to two-component regulatory system regulation.

References

[1] Battesti, A., and E. Bouveret. 2012. The bacterial two-hybrid system based on adenylate cyclase reconstitution in *Escherichia coli*. Methods 58:325-334.
[2] Halfmann, A., A. Schnorpfeil, M. Müller, P. Marx, U. Günzler, R. Hakenbeck, and R. Brückner. 2011. Activity of the two-component regulatory system CiaRH in *Streptococcus pneumoniae* R6. J. Mol. Microbiol. Biotechnol. 20:96-104.
[3] Marx, P., M. Meiers, and R. Brückner. 2015. Activity of the response regulator CiaR in mutants of *Streptococcus pneumoniae* R6 altered in acetyl phosphate production. Front. Microbiol. 5:772.
[4] Schnorpfeil, A., M. Kranz, M. Kovács, C. Kirsch, J. Gartmann, I. Brunner, S. Bittmann, and R. Brückner. 2013. Target evaluation of the non-coding csRNAs reveals a link of the two-component regulatory system CiaRH to competence control in *Streptococcus pneumoniae* R6. Mol. Microbiol. 89:334-349.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

519/GRP

Regulation of *rdh* Gene Transcription in *Dehalococcoides mccartyi* by MarR and Two-Component System Regulators

F. Greiner-Haas*[1], L. Krasper[1], C. Blumenscheit[1], U. Lechner[1]
[1]*Martin-Luther-Universität Halle-Wittenberg, Microbiology, Halle Saale, United Kingdom*

Dehalococcoides mccartyi strain CBDB1 is a strictly organohalide-respiring member of the Chloroflexi. The genome contains 32 different genes encoding reductive dehalogenase (*rdhAB*) homologues, reflecting its capacity to dehalogenate a broad range of halogenated compounds. Most *rdhAB* genes are associated with either MarR-type or two-component-system (TCS) regulators, suggesting a tight control of *rdhAB* transcription. Here, we demonstrate for one of the MarR regulators, Rdh2R, that it represses the transcription of three *rdhAB* genes (*cbdbA1453-54*, *cbdbA1455-54*, *cbdbA1598-97*). In addition, we studied the role of TCS 79-78, consisting of the histidine kinase (HK) CbdbA79 and the response regulator (RR) CbdbA78, in the transcriptional regulation of both the *rdhAB* (*cbdbA80-cbdbB3*) gene and the HK gene (*cbdbA79*).
D. mccartyi is not accessible to genetic manipulation. Therefore, the promoters of the *rdhA* genes and of the genes encoding the MarR or TCS regulators were transcriptionally fused to *lacZ* and each was integrated in single copy into the *E. coli* chromosome. Plasmids carrying *rdh2R* or the TCS-encoding genes *cbdbA79-78* were transformed into the reporter strains of interest. The promoter activity was assessed by beta-galactosidase assays. The interaction of Rdh2R with the respective *rdhA* promoters and its binding site was studied *in vitro* using electrophoretic mobility shift assays (EMSA) and analytical ultracentrifugation.

The results indicated activity of the promoters of the HK gene *cbdbA79* (P79) and of the *rdhA* gene *cbdbA80* (P80) suggesting their recognition by the RNA polymerase of the heterologous host. Within CBDB1 cultures, a subpopulation was found that contains a mutation in the RR-encoding gene *cbdbA78*. The mutation led to the exchange of a conserved arginine to methionine (R114M) within the dimerization domain. We have studied the influence of both the wildtype and the R114M TCS, on the activity of *P79* and *P80*. In addition, to simulate RR phosphorylation, a D55E mutation was introduced in both the wildtype and mutant RR and these were also tested. The results suggest that the unphosphorylated wildtype RR represses transcription of *cbdbA79*, whereas the D55E variant led to an increased *P79* activity, suggesting an activating function upon phosphorylation. In contrast, the *P80* promoter was positively influenced by the R114M D55E variant.
The purified MarR regulator Rdh2R interacted with a 13 bp direct repeat motif conserved in the promoter regions of the *rdhA* genes *cbdbA1453*, *cbdbA1455* and *cbdbA1598*. Rdh2R was dimeric in solution and a tetramer upon DNA-binding, which is unusual with respect to the short binding motif.
These findings suggest that *rdhAB* gene repression plays an important role in the lifestyle of *D. mccartyi*, probably controlling a subtle expression of specific *rdhA* genes in the presence of suitable compounds.

References
[1] Krasper *et al.* (2016) J. Bacteriol. 198, 3130-3141.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

520/GRP
Functional analysis of the YycH and YycI proteins in *Staphylococcus aureus*
M. Gajdiss*[1], J. Kienemund[1], G. Bierbaum[1]
[1]*University Clinics of Bonn, Institute for Medical Microbiology, Immunology and Parasitology, Bonn, Germany*

WalRK represents the only essential two-component regulatory system in the Gram-positive pathogen *Staphylococcus aureus* and plays a major role in cell wall metabolism [1]. Furthermore, WalRK has repeatedly been reported to be involved in the intermediate resistance of *S. aureus* to Vancomycin and to Daptomycin [2][3]. In *B. subtilis*, previous reports have suggested that the membrane-bound auxiliary proteins YycH and YycI act as negative regulators on the kinase WalK [4]. In a recent study with *S. aureus*, an activation of the WalRK regulon by YycH and YycI was reported [5]. To further investigate the regulatory role of the YycH and YycI proteins in *S. aureus*, the full-length recombinant proteins of the *wal* operon have been expressed and tested under *in vitro* conditions using detergent micelles and phospholipid-liposomes. In the *in vitro* assays, WalK autophosphorylation activity was stimulated by YycH and YycI and we could also show that the phosphatase activity of WalK on WalR is ADP-dependent. Furthermore, knock-down of the regulatory proteins YycH and YycI influenced autolysis and wall teichoic acid content. These results provide a deeper insight into the kinase/phosphatase equilibrium of WalK and suggest a positive regulatory role of the YycH and YycI proteins that differs from the one found in *B. subtilis*.

References
[1] Dubrac et al. (2007), *J. Bacteriol.* 189 (22), 8257–8269.
[2] Jansen et al. (2007), *Int. J. Med. Microbiol.* 297 (4), 205–215.
[3] Howden et al. (2011), *PLoS Pathog.* 7 (11), e1002359.
[4] Szurmant et al. (2007), *J. Bacteriol.* 189 (8), 3280–3289.
[5] Cameron et al. (2016), *Sci Rep.* 2016 Sep 7;6:30823

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

521/GRP
RNA-mediated thermoregulation of the cytotoxic necrotizing factor (CNF_Y) from *Yersinia pseudotuberculosis*
C. Twittenhoff*[1], F. Righetti[1], A. Nuss[2], S. Will[3], P. Stadler[3], P. Dersch[2], F. Narberhaus[4]
[1]*Ruhr University Bochum, Microbial Biology, Bochum, Germany*
[2]*Helmholtz Centre for Infection Research, Molecular Infection Biology, Braunschweig, Germany*
[3]*University of Leipzig, Department of Computer Science and the Interdisciplinary Center for Bioinformatics, Leipzig, Germany*
[4]*Ruhr-Universität Bochum, Microbial Biology, Bochum, Germany*

RNA thermometers (RNATs) are structured RNA elements that regulate translation in a temperature-dependent manner. At low temperatures, the mRNA forms a secondary structure that occludes the Shine-Dalgarno (SD) sequence and inhibits ribosome binding. Increasing temperature leads to melting of the structured mRNA, thus enabling its translation [1].
The foodborne pathogen *Yersinia pseudotuberculosis*, which causes a variety of gut associated diseases, rapidly adjusts its lifestyle and pathogenesis upon entry from external reservoirs into the warm-blooded host (37 °C) [2]. Translation of *lcrF*, coding for a transcriptional activator of several virulence factors, is controlled by a well-described RNAT [3].
A transcriptome-wide structure probing approach identified 16 novel RNAT from *Y. pseudotuberculosis* [4]. The newly discovered thermosensors are involved in virulence, metabolic adaptation, and other processes. One virulence related candidate is located in the 5 untranslated region (5 UTR) of *cnfY*, coding for the Yersinia cytotoxic necrotizing factor (CNF_Y). CNFγ is one essential virulence determinant in *Yersinia* as this toxin modulates inflammatory responses and protects the bacteria from attacks of innate immune effectors by activation of GTPases [5].
In this study, the regulatory potential of this RNAT was further validated. First, translational control by *cnfY* 5UTR was validated by reporter gene studies and Western blot analysis in *Escherichia coli* and *Y. pseudotuberculosis*. RNA structure probing of the potential thermosensor confirmed opening of the SD region upon increasing temperature. Furthermore, temperature-dependent binding of the 30S ribosomal subunit to the SD sequence was demonstrated by primer extension inhibition assay (toe printing assay). Insertion of stabilizing point mutations into the *cnfY* 5UTR led to impairment of the thermometer functionality. The obtained results suggest translational control of *cnfY* expression by an RNAT.

References
[1] Kortmann J and Narberhaus F (2012), *Nature Rev Microbiol*,10, 255-265.
[2] Nuss AM, Heroven AK, Waldmann B, Reinkensmeier J, Jarek M, Beckstette M and Dersch M (2015), PLoS Genet, 11(3).
[3] Böhme K, Steinmann K, Kortmann J, Seekircher S, Heroven AK, Berger E, Pisano F, Thiermann T, Wolf-Watz H, Narberhaus F and Dersch P (2012), *PLoS Pathog*, 8(2):e1002518
[4] Righetti F , Nuss AM, Twittenhoff C, Beele S, Urban K, Will S, Bernhart SH, Stadler PF, Dersch P , Narberhaus F (2016), *Proc Natl Acad Sci USA*, 113(26):7237-42.
[5] Schweer J, Kulkarni D, Kochut A, Pezoldt J, Pisano F, Pils MC, Genth H, Huehn J and Dersch P (2013), *PLoS Pathog,* 9(11).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

522/GRP

Phenotypic heterogeneity in *Photorhabdus luminescens* cell populations – a "bet-hedging" strategy?

S. Eckstein*[1], A. Langer[1], R. Heermann[1]
[1]*Ludwig-Maximilians-Universität München, Mikrobiologie, Martinsried/München, Germany*

Photorhabdus luminescens is an enteric Gram-negative bacterium that lives in mutualistic association with soil nematodes and is highly pathogenic for insect larvae. *P. luminescens* exists in two phenotypically different forms, designated as primary (1°) and secondary (2°) cells. Both cell types differ from each other in various phenotypic traits, most predominant is that 2° cells are less bioluminescent than 1°, non-pigmented and unable to live in symbiosis with the nematode partner. After prolonged cultivation single 1° cells undergo phenotypic switching and convert into 2° cells. However, the reason for phenotypic switching and the socio-biological aspect behind is unclear. Anyway, the switching process must be tightly controlled since a 100% switching frequency would lead to a complete breakdown of the bacteria´s life cycle. We therefore investigated whether the two cell types influence each other in phenotypic switching by signaling. However, cultivation of both cell types in the culture fluid of the respective other type did not result in an altered switching frequency. This led to the conclusion that neither the 1° nor the 2° cells control phenotypic switching of the respective other variant. However, competition assays of *P. luminescens* 1° and 2° cells in insect larvae as well as in culture revealed, regardless of the initial ratio of the two cell types, a growth advantage of 1° cells in the early exponential growth phase, whereas 2° cells overgrew the 1° cells in the stationary phase. We observed that 2° cells can better tolerate stress and nutrient limitation than 1° cells with respect to growth, whereas 1° cells are better adapted to a rich nutrient environment. In summary, our results give first evidence that phenotypic switching of *P. luminescens* is a "bet hedging" strategy, which ensures survival of the bacterial population after finishing the life cycle in the insect host. Though, the fate of the 2° cells in the soil remains to be elusive.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

523/GRP

What triggers transcriptional regulation of organohalide respiration in *Sulfurospirillum* ssp.?

J. Esken*[1], T. Goris[1], T. Schubert[1], G. Diekert[1]
[1]*University of Jena, Dept. of Applied and Ecological Microbiology, Jena, Germany*

Question: *Sulfurospirillum multivorans* is able to conserve energy via organohalide respiration (OHR). The key enzyme is the tetrachloroethene (PCE) reductive dehalogenase (PceA), an iron sulfur protein that harbors a cobamide cofactor (norpseudo-B12) at the active site. Recently, the genome sequence of the organism became available (1). The gene encoding PceA is located in close vicinity to a huge gene cluster for cobamide biosynthesis. The gene expression of *pceA* is positively controlled by PCE (2). In the absence of PCE the transcription ceased over an unusual high number of more than 100 generations (2). This observation raised the question for the signal, which drives an ongoing gene expression and production of functional PceA while PCE is absent. Since the formation of catalytically active enzyme is strictly dependent on the presence of cobamide, the role of the cofactor and the cobalt positioned in the center of the corrin ring in the regulation of OHR was investigated. In order to analyze the universality of the long-term phenotype among the reductively dehalogenating epsilonproteobacteria, *S. halorespirans* was included in this survey.
Methods: Both *Sulfurospirillum* isolates were repeatedly sub-cultivated with nitrate rather than PCE as terminal electron acceptor, while the type of cobamide cofactor produced in the cells was manipulated by guided cobamide biosynthesis. Additionally, the isolates were cultivated under cobalt limitation in order to determine its impact on gene regulation. The amount of PceA, the enzyme activity, the substrate conversion, and the transcript of the *pceA* gene was monitored. In addition, the level of cobamide cofactor in the cells was measured as well as the level of transcript of the cobamide biosynthesis genes.
Results: The gene equipment of *S. halorespirans* essential for OHR displays a high sequence identity compared to *S. multivorans*. Only a *tetR*-like repressor gene lost its function in *S. multivorans* due to an inserted transposase. However, in the absence of PCE, both isolates showed a long-term down-regulation of the OHR. Changes in the structure of the cofactor, which led to inactive PceA, did not influence the long-term regulation on transcriptional level. Limitations in cobalt supply also affected PceA activity. Whether the level of transcript is changed under these conditions is currently under investigation.
Conclusion: The catalytically active form of PceA does not trigger the regulation of OHR gene expression. From the modulation of cobalt concentration in the medium a decrease of the amount of cobamide in the cells is expected, which will allow for final conclusions on the role of the enzymes cofactor in the regulatory system. Furthermore, novel insights into the general role of cobalt in global gene regulation are envisaged.

References

[1] Goris, T. *et al.* (2014) *Environ Microbiol*. 16:3562-80
[2] John, M. *et al.* (2009) *J Bacteriol*. 191:1650-5

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

524/GRP

PerR-mediated response of *Clostridium difficile*

A. M. Michel*[1], M. Berges[1], D. Jahn[1]
[1]*TU Braunschweig, Institut für Mikrobiologie & BRICS, Braunschweig, Germany*

The bacterium *Clostridium difficile* is currently a major cause of high morbidity and mortality in Germany with several hundreds if not thousands patients dying per year. *C. difficile* associated diarrhea (CDAD) has caught increasing attention as one of the most deadly, infectious diseases. *C. difficile* is a strict anaerobic, Gram-positive bacterium which is able to form spores in an unfavorable environment. Little is known about the gene regulatory, protein and metabolic networks involved in the host associated life cycle of *C. difficile.* Our work originally aimed at the determination of the gene regulatory networks in response to peroxide stress. The peroxide repressor PerR from *Bacillus subtilis* is a member of the ferric uptake regulator (Fur) protein family and acts as a transcriptional repressor that senses H_2O_2 by metal-catalysed histidine oxidation [1]. To determine its functional role in *C. difficile* we constructed a deletion mutant of the clostridial *perR* homologue CD630_08260 using a group II intron based technology. In growth experiments no growth deficiency compared to the parental strain was monitored. Subsequently, the *perR* mutant was tested for its response to different stresses (e.g. H_2O_2, heat, acidic stress, starvation) using a systems biology approach with DNA microarrays and proteomics. These experiments indicated that PerR may contribute to the regulation of the survival under nutrient starvation stress conditions.

References

[1] Lee, J.-W. & Helmann, J. D. Biochemical characterization of the structural Zn_{2+} site in the *Bacillus subtilis* peroxide sensor PerR. *J. Biol. Chem.* 281**,** 23567–78 (2006).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

525/GRP

Regulation of methionine biosynthesis in *Staphylococcus aureus*: Impact of T-box riboswitch-mediated transcription control and RNA stability

F. Wencker*[1], S. Schoenfelder[1], W. Ziebuhr[1]
[1]*Universität Würzburg, Institut für Molekulare Infektionsbiologie, Würzburg, Germany*

N-formyl methionine is the universal N-terminal amino acid of prokaryotic proteins making methionine indispensable for bacterial growth. The common human pathogen *Staphylococcus aureus* is capable of synthesising methionine *de novo* and therefore to sustain in niches where the amino acid is lacking. Recently, we identified a unique hierarchical control pathway regulating *de novo* methionine biosynthesis in *S. aureus* involving stringent-response mediated CodY control in combination with a T-box riboswitch and RNA decay (1). Riboswitches are *cis*-acting RNA regulatory elements, located in 5-untranslated regions (5-UTRs) of genes. They typically undergo a conformational change upon interaction with a specific effector molecule, resulting in downstream gene expression control. The T-box family of riboswitches represent transcription termination control systems which bind uncharged cognate tRNAs as effector molecules. Interestingly, the T-box riboswitch residing in the 5-UTR of the *S. aureus metICFE-mdh* methionine biosynthesis operon specifically interacts with uncharged initiator $tRNA_i^{fMet}$, an unexpected finding whose exact function remains to be determined (1). By employing *in vitro* transcription of *met* leader RNA in presence of radioactively labelled tRNAs and electrophoretic mobility shift assays (EMSA), we identified in this study the nucleotides and regions critically involved in T-box leader RNA/tRNA interactions. In addition to T-box riboswitch-mediated transcription control, the *met* leader/*metICFE-mdh* mRNA was shown to undergo processing and rapid degradation involving various RNases (1). Here we demonstrate that stability of the *metICFE-mdh* mRNA varies over the length of the transcript with a longer lifespan towards the 3'-end of the transcript. From the combined data we hypothesise that targeted RNA decay might represent another level in the hierarchical methionine biosynthesis control network influencing translational efficiency and adjusting the protein amounts of the distinct enzymes of the pathway to current requirements.

(1) Schoenfelder, S. M.K. et al. 2013. Methionine Biosynthesis in *Staphylococcus aureus* Is Tightly Controlled by a Hierarchical Network Involving an Initiator tRNA-Specific T-box Riboswitch. Plos Pathogens 9(9).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

526/GRP

The role of the light-oxygen-voltage (LOV)-histidine kinase Dshi_1135 for regulation of the bacteriochlorophyll *a* biosynthesis in *Dinoroseobacter shibae*

M. Becker*[1], S. Heyber[1], E. Härtig[1], D. Jahn[1]
[1]*TU Braunschweig, Microbiology, Braunschweig, Germany*

Introduction: *Dinoroseobacter shibae* is a member of the Roseobacter group and belongs to the aerobic anoxygenic phototrophic bacteria (AAnP) which are using light in the presence of oxygen without producing oxygen to synthesize organic matter. *D. shibae* is using spheroidenone and bacteriochlorophyll *a* (Bchl*a*) as main light harvesting pigments [1]. Interestingly, bacteriochlorophyll *a* is synthesized in the dark and gets rapidly degraded under high light conditions [2]. During a transposon library screen, we identified the gene locus Dshi_1135 since inactivation of Dshi_1135 resulted in a complete loss of pigmentation.
Objectives: Analyses of the role of Dshi_1135 in light-dependent regulation of Bchl*a* biosynthesis in *D. shibae*.
Materials & methods: We used *in vivo* UV/Vis spectroscopy to identify mutants with altered absorption spectra compared to the wild type strain. We defined the Dshi_1135 regulon by comparing transcript levels of the regulatory mutant strain with the *D. shibae* wild type strain grown in the dark. Furthermore, we created a bchF-*lacZ* reporter gene fusion to analyse the gene expression in more detail. The LOV-HisKA protein Dshi_1135 was recombinantly produced in *E. coli* and purified for biochemical analyses.
Results: Dshi_1135 encodes a protein consisting of a potential light-oxygen-voltage (LOV) domain fused to a histidine kinase domain. It shows 42 % identity to a blue light-activated HK from *Erythrobacter litoralis* from which structural data are available [3]. Inactivation of Dshi_1135 resulted in a complete loss of Bchl*a* as shown by extraction and UV/Vis absorption measurement. Transcriptome analysis of the Dshi_1135 mutant strain compared to the wild type strain revealed loss of expression of the photosynthetic gene cluster. Additionally, in the *D. shibae* wild type strain $DFL12^T$ we found an increase of the *bchF-lacZ* reporter gene fusion.
Conclusion: The LOV-HisKA protein Dshi_1135 is part of signal transduction of the bacteriochlorophyll *a* biosynthesis regulation, presumably mediating blue light dependent activation.

References

[1] Buchan *et al.* (2005), Appl Environ Microbiol, 71(10): 5665–5677
[2] Tomasch *et al.* (2011), ISME J. 5: 1957-68
[3] Rivera-Cancel *et al.* (2014), PNAS 111(50): 17839-44

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

527/GRP

Combination of dRNA-seq and RIP-seq identifies two novel Hfq-dependent sRNAs in *Neisseria meningitidis* regulating the expression of a putative colonization factor

S. Bauriedl*[1], N. Heidrich[2], C. Schoen[1], J. Vogel[2]
[1]*Hygiene und Mikrobiologie Würzburg, Forschung, Würzburg, Germany*
[2]*Institute for Molecular Infection Biology, Würzburg, Germany*

Despite experimental evidence that differences in gene regulation among strains as well as the expression of small non-coding RNAs (sRNAs) affect meningococcal virulence [1-3], the organization of its transcriptome including in particular the biogenesis of sRNAs and their mode of action are only poorly understood so far.
Here, we used differential RNA sequencing (dRNA-seq) to uncover a single-nucleotide resolution map of the primary transcriptome of *Neisseria meningitidis* strain 8013. We further combined co-immunoprecipitation of sRNAs bound to the RNA chaperone Hfq with RNA sequencing (RIP-seq) to determine the set of Hfq-bound sRNAs along with their target mRNAs on a transcriptome-wide level. Using a green fluorescent protein based plasmid system we further validated the predicted interactions between selected sRNAs and their mRNA targets as indicated by Hfq RIP-seq analysis.
For the 1918 annotated protein coding sequences in strain 8013 dRNA-seq analysis predicted 1,625 transcriptional start sites (TSSs) with the majority of TSSs utilized in mid as well as late exponential growth in rich medium. The majority of the 706 primary TSSs (pTSSs) were generated for proteins with 382 pTSS obtained for single genes and 240 pTSSs obtained for genes located in operons. dRNA-seq further revealed 65 sRNAs of which 45 were not previously identified, and the expression of

over 20 was also confirmed by northern-blot analysis. By Hfq RIP-seq we could identify a large Hfq-centered post-transcriptional regulatory network comprising 24 validated sRNAs, and rifampicin treatment experiments demonstrated that Hfq binding confers enhanced stability on sRNAs. Using a green fluorescent protein based plasmid, we finally could verify that the methylcitrate lyase gene *prpB* (NMV_0472) which is considered to be involved in meningococcal colonization of the human nasopharynx [4] is post-transcriptionally repressed by the two structural highly similar sRNAs NMnc0017 and NMnc0018, thus indicating a central role of riboregulation in meningococcal life cycle.
In conclusion, this large expression compendium allows a deeper understanding of meningococcal transcriptome organization and riboregulation thus providing a valuable resource for the scientific community.

References
[1] Del Tordello E, et al. *J Bacteriol* 2012, 194**:**6217-6232.
[2] Fagnocchi L, et al. *PLoS One* 2015, 10**:**e0126325.
[3] Joseph B, et al. *J Bacteriol* 2010, 192**:**5363-5377.
[4] Catenazzi MCE, *et al*. *Mol Microbiol* 2014, 93:346-355

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

528/GRP
Discovery of RNA-protein complexes by Grad-seq
J. Hör*[1], K. Förstner[1,2], J. Vogel[1]
[1]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*
[2]*University of Würzburg, Core Unit Systems Medicine, Würzburg, Germany*

RNA-binding proteins (RBPs) are important factors in the regulation of gene expression. In bacteria, only two major families of regulatory RBPs are known: Hfq and CsrA, both of which act by regulating gene expression on mRNA level. While Hfq facilitates binding of small RNAs (sRNAs) to their target mRNAs and thereby leads to regulation, CsrA binds directly to mRNAs, altering their translation and stability. sRNAs are also a key factor in CsrA regulation, as they are able to sequester CsrA away from its mRNA targets (1). Furthermore, RBPs can be essential factors for bacterial virulence as it was shown for Hfq in the major human pathogen *Salmonella* Typhimurium (2). However, many bacterial species lack one or both of these two well-studied RBPs even though they express regulatory sRNAs, suggesting the existence of currently unknown RBPs (3).
Using gradient profiling by sequencing (Grad-seq), we are investigating the existence of overlooked RBPs (4). For this, whole bacterial cell lysates are run on a linear glycerol gradient, leading to partitioning of all soluble content. Since Grad-seq is performed under native conditions, RNA-protein complexes stay intact and sediment as a whole according to their biochemical properties. These interactions can then be investigated by fractionation of the gradient followed by RNA-seq and mass spectrometry of each of those fractions. The combined analysis of the resulting data sets allows us to draw conclusions which RNAs might interact with which proteins.

References
[1] Holmqvist E; Wright P. R; Li L; Bischler T; Barquist L; Reinhardt R; Backofen R; Vogel J. Global RNA recognition patterns of post-transcriptional regulators Hfq and CsrA revealed by UV crosslinking *in vivo*. *EMBO J* 2016, 35, 991-1011.
[2] Sittka A; Pfeiffer V; Tedin K; Vogel J. The RNA chaperone Hfq is essential for the virulence of *Salmonella typhimurium*. *Mol Microbiol* 2007, 63, 193-217.
[3] Jousselin A; Metzinger L; Felden B. On the facultative requirement of the bacterial RNA chaperone, Hfq. *Trends Microbiol* 2009, 17, 399-405.
[4] Smirnov A; Förstner KU; Holmqvist E; Otto A; Günster R; Becher D; Reinhardt R; Vogel J. Grad-seq guides the discovery of ProQ as a major small RNA-binding protein. *Proc Natl Acad Sci U S A*, 113, 11591-11596.

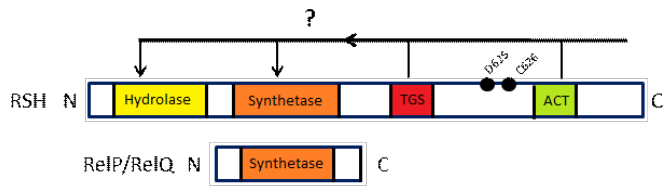
Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

529/GRP
The impact of RelP, RelQ and the functional domains of RSH on the stringent response in *Staphylococcus aureus*.
P. Horvatek*[1], F. Gratani[1], C. Wolz[1]
[1]*Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*

The stringent response is a global regulatory system activated under nutrient stress leading to growth arrest due to inhibition of replication, translation and re-programming of the transcriptome. The response is coordinated by (p)ppGpp (guanosin-tetraphosphate and guanosin-pentaphosphate) which is synthesized by activated RelA/SpoT homoloques (RSH). In *Stapyhlococcus aureus* there are additional small (p)ppGpp synthetases, RelP and RelQ, which are induced under cell wall stress conditions on the transcriptional level. The bifunctional RSH senses amino acid starvation presumably through recognition of unloaded tRNA at the A-site of the ribosome. RSHs are composed of a regulatory C-terminal domain and an enzymatic N-terminal domain with a hydrolase and synthetase domain (figure). Within the C-terminal domain conserved motifs such as TGS (**T**hreonyl-tRNA Synthetase, Obg family of **G**TPase, **S**poT), ACT (bacterial **A**spartat-Kinase, **C**horsimat-Mutase, Prephenat-Dehydrogenase **T**yrA) and a putative dimerization domain (DD) are present. The role of these domains for sensing, signal transduction and the switch between synthetase and hydrolase activity is largely unclear.
We analyzed the role of the C-Terminus of *S. aureus* RSH on the stringent response. Truncated and mutated versions of RSH were constructed and expressed in a (p)ppGpp^0 strain. As a read-out of stringent response we analyzed growth behavior and the expression of *rpsL* (coding for ribosomal protein S12, down-regulated under stringent conditions) and *brnQ1*/*aap* (coding for branched-chain amino acid transporters, up-regulated under stringent conditions)by Northern Blot analysis. RSH without C-terminus exhibited strong hydrolase but little synthetase activity in *S. aureus* and was unresponsive to stringent conditions imposed by mupirocin. Expression of the same construct in a *relA*/*spot* mutant of *E. coli* in contrast resulted in strong synthetase activity. The ACT domain of RSH was found dispensable for function in *S. aureus*. However, the TGS and DD domains are required for sensing of amino acid starvation and synthetase activity. In *S. aureus* the level of (p)ppGpp is detrimental for stress survival and growth control. Therefore, a fine tuning between degradation and synthesis is required for adaptation to different conditions.

Figure: Domain Organization of RSH and RelP/Q

Figure 1



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

530/GRP

Automated expansion of the extracytoplasmic function (ECF) σ factor protein family

D. Casas Pastor*[1], A. Goesmann[2], G. Fritz[1]
[1]*Philipps-Universität Marburg, LOEWE-Zentrum für Synthetische Mikrobiologie, Marburg, Germany*
[2]*Justus-Liebig-Universität Gießen, Gießen, Germany*

ECFs are the simplest members of the σ70 family of σ factors and are important players in bacterial responses towards environmental stress. Since their discovery, about 90 phylogenetically distinct ECF groups have been identified (1-3), enabling a group-specific study of their mechanism of action and making them applicable in synthetic biology. However, to date the classification of ECF groups has been limited to only about 500 organisms out of the more than 60,000 genomes currently annotated. This prompted us to develop a bioinformatic pipeline that allows for an automated and periodic update of the ECF family. To this end we designed a computational framework based on Hidden Markov Models (HMMs) that automatically extracts new ECFs from the current set of annotated genomes available at the NCBI database, and clusters them into new ECF groups. Moreover, HMMs of previously defined groups are automatically refined by using Receiver Operating Characteristic (ROC). As a result, the ECF library was expanded by more than 10-fold and was greatly enriched in proteins from previously under-represented phyla. Besides the identification of novel ECF groups, group-specific analysis of the genomic context of ECFs allows for a systematic analysis of their cognate anti-sigma factors, which have often co-evolved in the same operon.This work shows that the application of comprehensive data mining with periodical updates provides a powerful tool to identify new genetic elements in poorly represented phylogenetic classes. The extracted data provides the starting point for in-depth computational analyses of ECF-promoter and ECF-anti-σ interactions, as well as group-specific mechanisms of action.

References

[1] Staroń A, et al. (2009) The third pillar of bacterial signal transduction: classification of the extracytoplasmic function (ECF) sigma factor protein family. *Mol Microbiol* 74(3):557–581.
[2] Jogler C, et al. (2012) Identification of proteins likely to be involved in morphogenesis, cell division, and signal transduction in Planctomycetes by comparative genomics. *J Bacteriol* 194(23):6419–6430.
[3] Huang X, Pinto D, Fritz G, Mascher T (2015) Environmental sensing in Actinobacteria: a comprehensive survey on the signaling capacity of this phylum. *J Bacteriol* 197(15):2517–2535.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

531/GRP

Role of the stringent response for biofilm formation in *Staphylococcus aureus*

C. Kästle*[1], B. Kästle[1], D. Keinhörster[1], S. George[1], P. Horvatek[1], C. Wolz[1]
[1]*Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*

In *Staphylococcus aureus*, the stringent response can be activated by amino acid limitation or cell envelope damage. The subsequent production of alarmones (guanosine tetra- and pentaphosphate), collectively named (p)ppGpp, leads to growth inhibition and reprogramming at the genetic level. pppGpp is mainly synthesized by a RelA/SpoT homologue, RSH, with both synthase and hydrolase activity and a C-terminal sensory domain. The synthase activity is dependent on uncharged tRNA. Two additional small alarmone synthases, RelP and RelQ, showing only synthase activity are part of the cell wall stress stimulon and synthesize preferentially ppGpp. There is growing evidence that the stringent response is crucial for biofilm formation and maintenance in different organisms. Biofilm formation on both implanted medical devices and tissue protects the pathogen from the host immune response and antibiotic agents and therefore reduces treatment efficacy.
To investigate the influence of the stringent response on the formation of biofilm and how antibiotics affect this process we used different mutant strains lacking one, two or all three of the (p)ppGpp producing enzymes in *S. aureus* strain HG001 and USA300 JE2. These mutants lost the ability to induce the stringent response completely (triple mutant (p)ppGpp^0), in response to amino acid limitation (*rsh$_{syn}$*) or cell wall stress (*relP/relQ*). Biofilm formation was assessed in microtiter plates and structure was monitored by confocal microscopy using live/dead staining. Under non-inducing conditions no significant difference between mutant and wild type was detectable. However, addition of sub-inhibitory concentrations of cell wall active vancomycin significantly reduced biofilm formation in mutants lacking *relP* and *relQ*. The proposed (p)ppGpp inhibitory peptide DJK-5 showed biofilm inhibiting activity in wild type and mutant strains. This indicates that in *S. aureus* this anti-biofilm molecule acts independently of (p)ppGpp. Treatment of the biofilm with DNase, Proteinase or $NaIO_4$, revealed that the biofilm matrix is mostly composed of proteins and eDNA. Furthermore, the biofilm was only slightly dependent on the regulatory loci *sae*, *codY*, *agr* or *rot*. In summary, ppGpp produced by RelP or RelQ contributes to the maintenance of biofilms under cell wall stress conditions.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

532/GRP

Identification of contact sites between alternative σ factors and their anti-σ factors

A. Diehl*[1], S. Vecchione[1], D. Casas-Pastor[1], G. Fritz[1]
[1]*LOEWE Center for Synthetic Microbiology, Marburg, Germany*

In order to adapt to changing environments bacteria often take advantage of alternative s factors. The largest and most diverse group is the extracytoplasmic function (ECF) s family, whose members often react to external stimuli [1]. Signal transmission to the cytoplasm is achieved by regulation via membrane-spanning anti-s factors (ASs), which typically inactivate their respective ECF in the absence of a stimulus by sequestration. Despite significant heterogeneity in the periplasmic segment of the AS, a closer inspection of cytoplasmic segment shows a set of conserved motifs and domains, which cluster in phylogenetic groups similar to their corresponding ECFs. The most widespread feature is the so-called anti-σ domain I (ASD-I), which is responsible for binding the cognate ECF in more than 10 different ECF groups. To gain a better understanding of how these ASs specifically recognize their cognate ECFs, we investigate their contact sites by direct coupling analysis. This computational method predicts interacting amino acid residues on the basis of their co-evolution caused by compensatory mutations. We find that although the overall architecture of the binding interface is preserved, distinct contact motifs exist, that differ between ECF groups. Currently, we study the specificity of these ECF-AS interactions experimentally and explore whether exchanging these motifs can readily change specificity of AS groups. These results will be important for the rational design of synthetic circuits based on ECF σ factors, which rely on the precise and orthogonal regulation of ECF activity by anti-σ factors.

References
[1] Staroń, A. *et al.* (2009). Molecular Microbiology, 74(3), 557–581.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

533/GRP
The function of the ExxN phosphatase motif of the C4-dicarboxylate sensor kinase DcuS of *Escherichia coli* in signal transduction
S. Gencheva*[1], G. Unden[1]
[1]*Johannes Gutenberg University, Institute for Microbiology, Mainz, Germany*

The two component system DcuS-DcuR of *E. coli* controls expression in response to fumarate. It is composed of the membrane-bound histidine kinase DcuS and the cytoplasmic response regulator DcuR. Under anaerobic conditions DcuS forms a DcuS/DcuB sensor complex with the transporter DcuB. Complex formation is essential for conversion of DcuS to the C4-dicarboxylate responsive form and the activation of the kinase domain [1, 2]. *In vitro* studies show a positive effect of DcuS on DcuR dephosphorylation. The control of intercellular concentration of activated DcuR depends on its rates of phosphorylation and dephosphorylation. The DHp domain of DcuS contains a conserved ExxN phosphatase motif [3]. *In vivo* and *in vitro* studies were performed with ExxN variants to study the role of DcuS and DcuB in the control of phosphorylation and dephosphorylation.

References
[1] Unden, G., Wörner, S., Monzel, C. (2016) Adv Microbiol Physiol, 68:139–167.
[2] Worner, S. *et al.,* (2016) Environ Microbiol, doi: 10.1111/1462-2920.13418
[3] Huynh, T. N., Noriega, C.E., Stewart, V. (2010) Proc Natl Acad Sci U S A. 107: 21140–21145.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

534/GRP
Quorum quenching of *Photorhabdus spec.* cell-cell-communication by *Xenorhabdus szentirmaii*
J. Brehm*[1], S. Brameyer[1], R. Heermann[1], H. B. Bode[2]
[1]*Ludwig-Maximilians-Universität Munich, Biozentrum, Munich, Germany*
[2]*Goethe-Universität Frankfurt, Buchmann Institute for Molecular Life Sciences , Frankfurt am Main, Germany*

Photorhabus and *Xenorhabdus* species are Gram-negative bacteria that maintain a mutualistic association with specific soil nematodes, and are both highly pathogenic towards insect larvae. In nature both bacteria compete when their nematode partner would release them into one individual insect host. We therefore performed competition assays with insect larvae and co-infected them with both bacterial genera. Different mixtures of *X. szentirmaii* and *P. luminescens* or *P. asymbiotica* cultures, respectively, were injected into *Galleria mellonella* larvae and re-isolated after larvae death. Regardless of the initial ratios of the mixtures, *X. szentirmaii* out-competed both *Photorhabdus* species in the larvae. *P. luminescens* as well as *P. asymbiotica* both form cell clumps upon larva infection, which has been found to be an important virulence factor for both bacterial species, probably as a protection mechanism against the innate immune system of the insect. Cell clumping is in both organisms under control of a LuxR-based quorum sensing[1,2]. In *P. luminescens* the LuxR-like receptor PluR detects photopyrones as quorum sensing molecules, the homologous receptor PauR of *P. asymbiotica* senses dialkylresorcinols. For competing organisms the LuxR-like receptor is a common target to interrupt cell-cell communication of competitors, a process termed quorum quenching (QQ). We therefore tested a library of different secondary metabolites (HB) of *X. szentirmaii* and other *Xenorhabdus* bacteria in their capacity to act as agonists or antagonists onto the PluR- and PauR-based quorum sensing systems, respectively. Some compounds specifically influenced the PluR-based but not the PauR-based quorum sensing system. While molecules HB1, HB2 and HB11 showed enhanced PluR-mediated reporter gene activity, the structures HB97 and HB121 led to an interception of the reporter gene expression. Therefore, QQ might be one of the strategies how *X. szentirmaii* might out-compete *Photorhabdus* species upon a co-infection in nature. In summary, we identified specific QQ molecules for photopyrone-mediated quorum sensing systems. These QQ compounds might be interesting candidates for novel antimicrobial drugs.

References
[1] Brameyer S, Kresovic D, Bode HB, Heermann R. (2015). Dialkylresorcinols as bacterial signaling molecules. Proc Natl Acad Sci U S A. 2015 Jan 13;112(2):572-7
[2] Brachmann AO, Brameyer S, Kresovic D, Hitkova I, Kopp Y, Manske C, Schubert K, Bode HB, Heermann R. (2013) Pyrones as bacterial signaling molecules. Nat Chem Biol. Sep;9(9):573-8

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

535/GRP
The *Acinetobacter* DNA-(adenine N6)-methyltransferase AamA – an epigenetic regulator –
U. Blaschke*[1], B. Suwono[1], E. Skiebe[1], S. Fuchs[1], G. Wilharm[1]
[1]*Robert Koch-Institut, Wernigerode, Germany*

Epigenetics deals with heritable changes in gene expression without any changes in the DNA sequence. In bacteria the most studied epigenetic mechanism is DNA methylation which is carried out by DNA methyltransferases (MTases) [1,2]. These enzymes transfer methyl groups from S-adenosyl-L-methionine (SAM) to adenine or cytosine bases. This process protects DNA against digestion from restriction endonucleases and is important for the regulation of various physiological processes such as mismatch repair and transcription with an impact on virulence as well as motility [3,4]. The *Acinetobacter baumannii* genome encodes a DNA-(adenine N6)-methyltransferase, designated A1S_0222 in strain ATCC 17978 and seems to act without a corresponding endonuclease. We hypothesized the DNA adenine methyltransferase A1S_0222 to impose epigenetic control in *Acinetobacter baumannii* and approached its characterization.
Making use of the naturally competent *A. baumannii* strain 29D2 we inactivated the gene A1S_0222 (29D2 *aamA::Km*). Single-molecule real-time (SMRT) sequencing was performed on 29D2 wildtype and the A1S_0222 mutant for a comparative methylation pattern analysis.
The recognition site of DNA adenine methyltransferase A1S_0222 could be identified as GAATTC. After SMRT sequencing, data were screened for m6A motifs. Inactivation of A1S_0222 resulted in the loss of the specific methylation pattern. Furthermore, the phenotypic characterization illustrates a deficiency in surface-associated motility of 29D2 *aamA::Km* and an increased susceptibility to the tested antibiotics compared to the wildtype. The mutant also shows an attenuation in the *Galleria mellonella* infection model.
Taken together, the results demonstrate that A1S_0222 encodes a DNA adenine-methyltransferase which methylates the GAATTC sequence. Moreover, SMRT sequencing reveals differences in the methylation pattern of 29D2 wildtype compared to the *aamA* mutant which may explain different phenotypic appearance.

References
[1] Cheng X. 1995. Structure and function of DNA methyltransferases. Annual review of biophysics and biomolecular structure 24:293-318
[2] Bart et al., 2005. Direct detection of methylation in genomic DNA. Nucleic Acids Res 33:e124
[3] Wion and Casadesús, 2006. N6-metyl-adenine: an epigenetic signal for DNA-protein interactions. Nature Reviews Microbiology. 10.1038/nrmicro1350
[4] Adhikari S. and Curtis P.D., 2016. DNA methyltransferases and epigenetic regulation in bacteria. FEMS Microbiol Rev. 2016 Sep;40(5):575-91

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

536/GRP

Comparative expression of Iron Super Oxide Dismutases (FeSOD A/ B) genes in sensitive and resistant field isolates of L. tropica to meglumine antimoniate using Real-Time RT-PCR

H. Hajjaran*[1], E. Kazemi-Rad[2], M. Mohebali[1], R. Raoofian[3], M. A. Oshaghi[4]
[1]*Tehran University of Medical Sciences, Medical Parasitology and Mycology, Tehran, Islamic Republic Of Iran*
[2] *Pasteur Institute of Iran, Medical Parasitology, Tehran, Islamic Republic Of Iran*
[3]*Legal Medicine Organization, Legal Medicine Research Center, Tehran, Islamic Republic Of Iran*
[4]*Tehran University of Medical Sciences, Medical Entomology and Vector Control, Tehran, Islamic Republic Of Iran*

Background: Kinetoplastids family, including Leishmania species for survival in the host macrophages must be defense against the free radicals. Iron-Superoxide dismutase (Fe-SOD) is an antioxidant enzyme contributing to radical super oxidase dismutation to prevent cellular oxidative damage.
Methods: In this comparative study, we analyzed the expression level of Iron superoxide dismutase mitochondrial (SOD A) and glycosomal (SOD B) in 26 meglumine antimoniate (Glucantime®) healing (sensitive) and non-healing (resistant) Leishmania tropica field isolates (Leishmania tropica isolates from responsive and unresponsive patients). Sensitive and resistant L. tropica parasites were isolated from anthroponotic cutaneous leishmaniasis (ACL) patients. After RNA extraction and cDNA synthesis, Real-Time RT-PCR approach was utilized to investigate the relative expression level of resistant and sensitive field isolates with respect to the standard isolate.
Results: Real time RT-PCR revealed a significant down regulation of SOD A (2.81 Fold) in resistant isolates, whereas SOD B was upregulated (4.01 fold) in resistant isolates compared to sensitive ones ($P<0.05$).
Conclusions: Our primary results suggest that alteration in the level of superoxide dismutases expression in resistant parasites could potentially contribute to detoxify reactive radicals and thus protects cell from antimony-induced oxidative stress. Moreover, since SOD B was up regulated in all resistant L. tropica isolates, it could be considered as potential biomarkers for monitoring of clinical resistant isolates.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

537/GRP

tRNA modifications in *Pseudomonas aeruginosa*

S. Grobe*[1], S. Doberenz[1], M. Schniederjans[2], V. Kaever[3], S. Häußler[2,1]
[1]*TWINCORE, Centre for Experimental and Clinical Infection Research GmbH, Molecular Bacteriology, Hannover, Germany*
[2]*Helmholtz Centre for Infection Research, Molecular Bacteriology, Braunschweig, Germany*
[3]*Research Core Unit Metabolomics, Hannover Medical School, Hannover, Germany*

Introduction: Transfer ribonucleic acids (tRNAs) are the most post–transcriptionally modified RNA. The biological functions of many tRNA modifications are still not understood, but it is known that they are critical for the tRNA structure and function affecting the efficiency and fidelity of translation. At the global level, tRNA modifications are proposed to function as translational regulators in pathways such as stress response and virulence [1].
The opportunistic pathogen *Pseudomonas aeruginosa* exhibits a huge repertoire of complex adaption strategies. Besides transcriptional control also translational regulation plays an important role for these processes [2]. Modified ribonucleosides in tRNA and their impact on the translational regulation in *P. aeruginosa* are still being determined.
Objectives: We investigate tRNA modifications of *P. aeruginosa* with the overall aim to obtain a modification map of the individual tRNAs and to unravel the impact of those modifications on the translational efficiency.
Materials & methods: For this purpose we have established a protocol in our lab to isolate individual tRNAs of *P. aeruginosa* PA14 that are purified by the use of tRNA specific DNA-oligonucleotides that are coupled to streptavidin coated beads. After confirming the purity of the individual tRNAs by Illumina Sequencing, they are analyzed by LC-MS/MS to identify modified ribonucleosides.
Results: Multiple sequence alignment with all 38 PA14 tRNAs showed a conserved sequence at the 5' and 3' end, with less homology in the anticodon stem-loop (ASL). To ensure tRNA specificity when purifying individual tRNAs, we designed the DNA-oligonucleotides complementary to the ASL. We successfully isolated the individual $tRNA^{Asp(GUC)}$ of PA14. LC-MS/MS analysis revealed five modified ribonucleosides in this individual tRNA, namely 5-methyluridine (m5U), 7-methylguanosine (m7G), 2-methyladenosine (m2A), Dihydrouridine (D) and Queuosine (QtRNA).
Conclusion: We determined five modified ribonucleosides present in the purified PA14 $tRNA^{Asp(GUC)}$.
For more detailed analysis we will not only identify the modification present in a specific tRNA but to also precisely localize the modification within the tRNA sequence. For this purpose LC-MS/MS analysis on partially digested tRNAs are planned. To correlate the LC-MS/MS data to a biological function, further investigations are needed. The link of tRNA modifications to *P. aeruginosa* phenotypes will be determined by characterizing mutants lacking a specific tRNA modifying enzyme. We will also perform Ribosome Profiling of these mutants to investigate the impact of tRNA modifications on the translational process.

References
[1] El Yacoubi et al. 2012, *Annu Rev Genet.*
[2] Coggan et al. 2012, *Curr Issues Mol Biol.*

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

538/GRP

Studying extracellular Phr-peptide signaling in *Bacillus subtilis* by FRET

H. Babel*[1,2], V. Sourkjik[1], I. B. Bischofs[1,2]
[1]*Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany*
[2]*University of Heidelberg, Center for Quantitative Analysis of Cellular and Molecular Biosystems (BioQuant), Heidelberg, Germany*

Extracellular peptides control important developmental processes in Gram-positive bacteria. One class of peptides is sensed extracellularly by histidine kinases and serves in quorum sensing. Others are sensed intracellularly by receptors from the RNPP-family and their function is less clear. For example, the PhrA-pentapeptide, which controls sporulation via the RapA-receptor in *B. subtilis*, was suggested to be part of a *cis-acting* timing device rather than acting as a *trans-acting* signaling molecule based on the failure of complementation experiments with cell-free supernatants.
Here we developed a novel FRET-reporter system, based on the interaction of the RapA-receptor with the Spo0F-regulator, to quantitatively elucidate different aspects of PhrA-signaling with the help of acceptor-photobleaching experiments.
As expected, the RapA-receptor and Spo0F-regulator interact in *Bacillus subtilis*. When cells were stimulated with different peptides they show a selective FRET-response to PhrA. Dynamic measurements of the intra- and extracellular peptide-processing indicate that cells completely internalize PhrA within minutes to saturate the receptor. Moreover, the active uptake of the signaling peptides by oligo-permeases allows cells to amplify a nM extracellular signal into a µM intracellular signal, which could explain the low affinity of Rap-receptors that was measured *in vitro*. Finally we show that the cell-free supernatant of sporulating cells contains low but sufficient amounts of PhrA to switch the FRET-reporter system.
Our data implies that PhrA could act as *trans* acting signaling molecule and we suggest that signal amplification could play an important role in intracellular peptide signaling.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

539/GRP

Investigating the effect of a *cis*-regulatory element on the glycine-dependent regulation of a putative amino acid symporter gene in *Streptococcus pyogenes*

A. Khani*[1], N. Popp[1], R. Pappesch[1], B. Kreikemeyer[1], N. Patenge[1]
[1]*Rostock University, Institute of Microbiology, Virology and Hygiene, Rostock, Germany*

Introduction: Riboswitches are elements found in the 5′-untranslated region (UTR) of mRNAs that exert their regulatory control over the transcript in a *cis*-fashion by directly binding a small molecule ligand. In a genome-wide approach to identify sRNA-candidate genes in the group A *Streptococcus pyogenes* M49 591 (GAS M49), a number of potential *cis*-regulatory elements was detected1.
Objective: In this study, we investigate the glycine-dependent gene expression regulation by a *cis*-regulatory element (*ribogly*) located in the 5"-UTR of a putative sodium-alanine symporter gene in *S. pyogenes* M49.
Materials & Methods: Gene expression was tested using a luciferase (LUC) reporter gene system. The *ribogly* fragment from GAS M49 was fused to the *luc* reporter gene carried by pFW11_*luc2*. GAS M49 was transformed with the resulting plasmid and the construct was inserted into the genome. Bacteria were grown under varying conditions and LUC activity was measured over time. Transcript analyses were performed by RT-qPCR.
Results: Growth of GAS M49 in CDM w/o glycine (Gly) was poor. Maximal LUC activity was detected after 3 h at 0.01 mM Gly. At 10 mM Gly no LUC induction was detected. There was no LUC induction by either serine or alanine. Transcription of the *ribogly* fragment as well as the full-length putative symporter gene increased upon addition of 0.01 mM Gly. To investigate whether the *ribogly* promoter affects the induction behavior it was replaced with a foreign promoter (*groE*).
No induction was observed with the foreign promoter in the presence of the *ribogly*. Furthermore, the *ribogly* promoter was fused directly to the reporter gene. Again, no induction by Gly could be detected. Stability of the putative riboswitch and symporter gene transcripts was investigated during induction. Full length symporter gene mRNA stability was significantly higher in the presence of 0.01 mM Gly compared to 10 mM. In contrast, the putative riboswitch transcript decayed rapidly at both concentrations.
Conclusions: The sequence and secondary structure of the *ribogly* element in GAS M49 are homologous to the glycine riboswitch in *B. subtilis*. In *B. subtilis*, glycine binding induces anti-termination by conformational changes of the riboswitch. We show a glycine-specific response at low concentrations (0.01-0.1 mM Gly) in GAS M49. However, anti-termination was not seen in GAS M49 at different Gly concentrations. Stability and potential processing of the mRNA needs to be studied in detail by Northern blot analyses.

References

[1] Nadja Patenge, André Billion,Peter Raasch & Jana Normann (2012) Identification of novel growth phase- and media-dependent small non-coding RNAs in *Streptococcus pyogenes* M49 using intergenic tiling arrays. *BMC Genomics,*13, 550

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

540/GRP

Functional characterization of a processed antisense sRNA pair of the food-borne pathogen *Campylobacter jejuni*

S. Svensson*[1], M. Alzheimer[1], G. Dugar[1], C. Sharma[1]
[1]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*

High-resolution transcriptome mapping has identified several candidate sRNAs in *C. jejuni* (1), a significant foodborne pathogen, as well as in the related gastric pathogen *Helicobacter pylori* (2). These sRNAs represent an uncharacterized layer of post-transcriptional regulation that might control pathogenesis. In addition, because *C. jejuni* and *H. pylori* both lack homologs of bacterial proteins central to RNA metabolism, such as Hfq and RNase E, the biogenesis/activity of these sRNAs might also use novel mechanisms. In fact, characterization of *H. pylori* RepG revealed a novel mechanism by which an sRNA directly targets a length-variable G-repeat in a target mRNA 5"UTR, linking phase variation to post-transcriptional regulation (3). Here, we are characterizing a pair of overlapping antisense sRNAs of *C. jejuni*, CJ-RepG and CJ-RepGAS. Both are processed by RNase III, and the most abundant species in wild-type is a 70-nt sRNA with a C-rich loop derived from the 3" end of the CJ-RepG primary transcript. Processing of CJ-RepGAS, but not of CJ-RepG, requires its antisense partner. Deletion of the sRNA locus leads to upregulation of *ptmG*, which encodes an enzyme in the legionaminic acid flagellin-glycosylation pathway, at both the mRNA and protein level. Regulation in the deletion strain can be complemented by either CJ-RepG alone (without its partner CJ-RepGAS) or by HP-RepG. CJ-RepG and HP-RepG directly interact with a G-rich sequence near the ribosomal binding site of *ptmG* mRNA, suggesting an analogous regulatory mechanism. In-depth characterization of CJ- and HP-RepG–target interactions may uncover what determines target specificity in C-rich sRNAs, which are also present in more distant bacteria and can be Hfq-

independent. Moreover, understanding the biogenesis of CJ-RepG and its antisense partner will also provide insight into RNase E-independent mechanisms of sRNA processing, as well as how sRNAs themselves might be post-transcriptionally regulated. Finally, both CJ-RepG and its target *ptmG* affect *C. jejuni* interactions with epithelial cells in a novel tissue-engineered model for bacterial intestinal infection, indicating that post-transcriptional control can in fact impact pathogenesis.

References

[1] Dugar G, Herbig A, Förstner KU, Heidrich N, Reinhardt R, Nieselt K, Sharma CM (2013). High-resolution transcriptome maps reveal strain-specific regulatory features of multiple *Campylobacter jejuni* isolates. PLOS Genetics 9(5):e1003495.
[2] Sharma CM, Hoffmann S, Darfeuille F, Reignier J, Findeiß S, Sittka S, Chabas S, Reiche K, Hackermüller J, Reinhardt R, Stadler PFand Vogel J (2010). The primary transcriptome of the major human pathogen *Helicobacter pylori.* Nature 464, 250-255.
[3] Pernitzsch SR, Tirier SM, Beier D, Sharma CM (2014). A variable homopolymeric G-repeat defines small RNA-mediated posttranscriptional regulation of a chemotaxis receptor in *Helicobacter pylori*. PNAS 111(4):E501-10.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

541/GRP

Salmonella promotes infection by decreasing host cell E2F1 expression

C. Aguilar*[1], C. Maudet[1], A. Eulalio[1]
[1]*Institute for Molecular Infection Biology, Host RNA Metabolism Group, Würzburg, Germany*

Introduction: A key determinant for the establishment of productive infection by intracellular pathogens is their ability to subvert host cellular processes in order to ensure invasion and replication. In a recent study, we have shown that *Salmonella* is able to downregulate the expression of several microRNAs (miRNAs; e.g. miR-15 family), in both infected and bystander cells, in order to promote infection. Interestingly, some of these miRNAs have been shown to be under the control of the transcription factor E2F1. The main goal of the present work is to characterize the mechanism by which E2F1 expression is regulated in *Salmonella* infected and bystander cells.
Material and Methods: HeLa cells were infected with *Salmonella enterica* serovar Typhimurium. Cells were treated with conditioned medium (CM) from *Salmonella* infected cells, tunicamycin, DTT and TUDCA prior to infection. siRNAs targeting E2F1 were used to knockdown expression of E2F1 prior to infection. Comparative proteomic analysis of CM from *Salmonella* and mock infected cells was performed by LC-MS/MS.
Results: We found that E2F1 expression is decreased in both *Salmonella*-infected and bystander cells in a time and MOI dependent manner. Treatment of naïve HeLa cells with CM from *Salmonella* infected cells resulted in a decrease in E2F1 protein levels, and consequently in a lower expression of miRNAs under the control of this transcription factor (e.g. miR-15, let-7i-3p). The treatment of the CM with RNAse, DNAse or proteinase K revealed that the signal responsible for E2F1 downregulation is a protein. Proteomic analysis of the CM identified a set of proteins involved in the induction of endoplasmic reticulum (ER) stress. Accordingly, we found an increase of BIP/GRP78 protein, a master regulator of the unfolded protein response that is strongly upregulated upon ER stress, in *Salmonella* infected cells as well as in CM treated cells, indicating an increase of ER stress in these conditions. Importantly, decrease of E2F1 expression was observed in epithelial cells after treatment with ER stress inducers (e.g. DTT, tunicamycin), which was translated into an increase of bacterial invasion and replication in bystander and infected cells, respectively. Similar results were observed upon E2F1 knockdown using siRNAs or treatment with CM. In contrast, a significant reduction of *Salmonella* infection was observed after treatment with the ER stress inhibitor TUDCA.
Conclusion: Our results demonstrate that *Salmonella* induces ER stress in infected and bystander cells, which results in decreased expression of the transcription factor E2F1. Importantly, E2F1 regulates the expression of miRNAs that have been shown to be inhibitory for *Salmonella* infection. Overall, we have uncovered a novel mechanism whereby *Salmonella* renders host cells more permissive to infection through the regulation of E2F1 expression and downstream microRNA network.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

542/GRP

Integrated network of different sigma factors, two-component systems and non-coding RNAs that regulate transcription of the *rpoE* gene encoding ECF sigma factor in *Escherichia coli* in response to different signals

G. Klein*[1], A. Stupak[1], P. Wojtkiewicz[1], D. Biernacka[1], S. Raina[1]
[1]*Gdansk University of Technology, Unit of Bacterial Genetics, Gdansk, Poland*

The RpoE sigma factor is essential for the viability of *Escherichia coli*. RpoE regulates extracytoplasmic functions including several key virulence factors in pathogenic bacteria. Among the essential factors that RpoE regulates include critical steps in lipopolysaccharide (LPS) translocation and some of its non-stoichiometric modifications. These non-stoichiometric modifications play critical role in survival under adverse conditions and resistance to antibiotics. The essential RpoH-regulated heat shock proteins FtsH and LapB tightly regulate the first committed step in the LPS biosynthesis by controlling the turnover of LpxC. Severe defects in the LPS biosynthesis also induce RpoH and RpoE regulons. However, the mechanism of sensing LPS defects that induce *rpoE* transcription is poorly understood. Transcription of the *rpoE* gene is positively autoregulated by $E\sigma^E$ and by additional mechanisms that control the expression of its distally located promoter(s). Mapping of 5′ ends of *rpoE* mRNA identified five new transcriptional initiation sites (P1 to P5) located distal to the $E\sigma^E$-regulated promoter. These promoters are activated in response to unique signals. Out of these P2, P3 and P4 defined major promoters, recognized by RpoN, RpoD and RpoS sigma factors, respectively. Isolation of *trans*-acting factors, *in vitro* transcriptional and gel retardation assays revealed that the RpoN-recognized P2 promoter is positively regulated by the QseE/F two-component system and NtrC activator, while the RpoD-regulated P3 promoter is positively regulated by the Rcs system in response to defects in LPS core biosynthesis, overproduction of certain lipoproteins and by the global regulator CRP. Strains synthesizing Kdo_2-LA LPS exhibited up to 7-fold increase in the *rpoE*P3 activity, which was abrogated in Δ(*waaC rcsB*). Overexpression of a novel 73 nt sRNA *rirA* (RfaH interacting RNA) generated by the processing of 5′UTR of the *waaQ* mRNA induces the *rpoE*P3 promoter activity concomitant with a decrease in LPS content and an impaired *O*-antigen incorporation. In the presence of RNA polymerase, RirA binds LPS regulator RfaH known to prevent premature transcriptional termination of *waaQ* and *rfb* operons. Experimental evidence supports a model of titration of RfaH by RirA leading to LPS defects and the activation of *rpoE* transcription. The RpoS-regulated *rpoE*P4 promoter is induced upon entry into stationary phase and its activity is modulated by factors that control RpoS amounts/stability.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

543/GRP

Regulation of LPS composition, biosynthesis and critical checkpoints in its assembly by non-coding regulatory RNAs

S. Raina*[1], G. Klein[1]
[1]*Gdansk University of Technology, Unit of Bacterial Genetics, Gdansk, Poland*

In *Escherichia coli*, alternative sigma factors (RpoE and RpoH), transcriptional factor RfaH, envelope stress responsive two-component systems and small non-coding RNAs control several steps of lipopolysaccharide (LPS) biosynthesis, its assembly and nonstoichiometric modifications. The outer membrane is asymmetric in composition due to the restricted localization of LPS in the outer leaflet while phospholipids face the periplasm. *In vivo* amounts and ratio of LPS and phospholipids are tightly regulated and any imbalance leads to bacterial cell death. LPS is essential for the bacterial viability as it provides the permeability barrier and is the major virulence determinant in pathogenic bacteria. Non-coding regulatory RNAs control LPS biogenesis at several steps that include availability of metabolic precursors like UDP-GlcNAc (GlmY/GlmZ), lipid A and inner core modifications (MicA, MgrR and RybB), synthesis of glycoforms with either two or three Kdo residues (RybB) and expression of long *waa* and *rfb* operons that contain genes whose products are involved in LPS core and *O*-antigen biosynthesis (RirA). LPS is extremely heterogeneous and dynamically altered in response to different stresses. Thus, induction of the *rpoE* gene leads to a switch to the synthesis of usually rare LPS glycoform that carries a truncation in the outer core with a concomitant incorporation of a third Kdo. This switch is controlled by RpoE-regulated RybB sRNA and by ppGpp. RpoE-transcribed sRNAs regulate major components of the outer membrane like OMPs (RybB, MicA and SlrA) and LPS (RybB and MicA). However, SlrA controls synthesis of the major lipoprotein Lpp and hence the phospholipid availability. Among these sRNAs, RirA is located in the 5′UTR and is generated by the processing of *waaQ* mRNA. The RirA sRNA was identified while studying the regulation of the *rpoE* gene and processes that control LPS amounts. RirA acts by binding to RfaH, thereby titrating RfaH availability, leading to decrease in the transcription of LPS biosynthetic operons containing *ops* pause site (binding site for the RfaH transcription factor). This RirA-mediated regulation of RfaH could serve as a checkpoint for control of LPS amount. Furthermore, we also show that Qse (Glr) and Cpx two-component systems and GcvB sRNA are also involved in modulating LPS structural alterations. Analysis of lipid A of *micA* and *mgrR* deletion derivatives revealed absence of P-EtN modification, revealing additional controls by such sRNAs.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

544/GRP

Identification of a [3Fe-4S]$^{1+}$ cofactor and functional analysis of the iron responsive regulator RirA from *Dinoroseobacter shibae*

M. Behringer*[1], E. Härtig[1], D. Jahn[1]
[1]*TU-Braunschweig, Microbiology, Braunschweig, Germany*

Introduction: In many bacteria iron dependent regulation is mediated by the global ferric-uptake regulator Fur and the iron responsive regulator Irr. The *Dinoroseobacter shibae* genome exhibits genes encoding Fur and Irr regulators and in addition, a gene encoding a protein homolog of the rhizobial iron regulator RirA.
Objectives: The rhizobial iron regulator RirA from *D. shibae* belongs to the Rrf2- family of transcription factors and is supposed to coordinate a Fe-S cluster and thereby measure iron availability [1].
Materials & Methods: A Δ*rirA* knockout mutant strain was created to analyze the role of the regulator in adaptation to iron limitation. To complement the Δ*rirA* mutant strain, a construct with a N-terminal StrepII-tag and a constitutive promoter was used. RirA protein fused with a StrepII-tag was recombinantly produced and purified under anaerobic conditions. UV/Vis and electron paramagnetic resonance (EPR) spectroscopy as well as whole cell Mössbauer analyses were used to determine the nature of the Fe-S cluster. The Fe content of the protein was determined with the atom absorbance spectroscopy (AAS). Four cysteine residues of RirA were changed to alanine via site directed mutagenesis of the corresponding gene. DNA binding of the anaerobically purified RirA wildtype was analyzed using electro mobility shift assays (EMSA).
Results: The Δ*rirA* mutant strain showed a reduced growth under iron limitation compared to the wild type strain indicating a role in iron-dependent gene regulation. Complementation of the Δ*rirA* mutant strain was successful since the complemented strain grew like the wild type strain. Analyses of anaerobically purified wildtype RirA using UV/Vis spectroscopy revealed an absorption shoulder at 420 nm, typical of Fe-S cluster containing proteins. Using EPR and Mössbauer spectroscopy a [3Fe-4S]$^{1+}$ cluster was identified. AAS measurements of iron, resulting in a 3:1 ratio of iron per molecule RirA, supported [3Fe-4S]$^{1+}$ as cofactor. Since three of the four cysteine mutants of RirA showed a drastically reduced absorption in UV/Vis spectroscopy these cysteines may serve as ligands for [3Fe-4S]$^{1+}$ cluster. Using EMSA analyses, binding of RirA to *hemB2* promoter sequences was shown.
Conclusion: The rhizobial iron regulator RirA from *D. shibae* is coordinating an unusual [3Fe-4S]$^{1+}$ cluster as cofactor and is thereby different to other iron-dependent regulators known so far. Mutation of RirA in *D. shibae* caused a growth defect which implicates a role in iron-dependent gene regulation.

References

[1] Bhubhanil, S, Niamyim, P, Sukchawalit, R, and S. Mongkolsuk (2013). Cysteine desulphurase-encoding gene *sufS2* is required for the repressor function of RirA and oxidative resistance in *Agrobacterium tumefaciens*. *Microbiology*, 160:79-90

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

545/GRP

Machine learning for cross-species RNA-protein interaction prediction

B. Beckmann*[1], A. Bressin[2], D. Figini[1], A. Marsico[2]
[1]*Humboldt Universität zu Berlin, IRI Life Sciences, Berlin, Germany*
[2]*Max-Planck Institute for Molecular Genetics, Berlin, Germany*

RNA-binding proteins (RBPs) play major roles in post-transcriptional regulation of gene expression, e.g. as key proteins in RNA-transport or translation. Particularly in eukaryotic species, discovery of RNA-protein complexes (RNPs) has been driven by high-throughput techniques such as CLIP-Seq or mRNA interactome capture, leading to identification of novel interactions between RNA and proteins. However, while CLIP-type approaches have been performed in prokaryotes successfully, identification of RBPs in bacteria remains a challenge to date.
Here, we present TriPepSVM, a machine learning approach implementing a support vector machine (SVM) to predict RBPs from a taxon or single species. The SVM is trained solely on i) tripeptides (= amino acid triplets) and ii) the fraction of unstructered regions in RBPs and non-RBPs.
We tested our novel machine learning algorithm first on established human datasets containing >1000 well established

RBPs. Surprisingly and despite its simple training set, TriPepSVM outperforms other prediction tools based on RNA-binding domain similarity in performance tests. Next, we searched for RBPs in bacterial species in which, on average, RBPs are not as well characterised due to technical difficulties to identify mRNA-interacting proteins in prokaroytes that lack poly(A) tailing. We identify >90% of the known RBPs in *Salmonella* Typhimurium and predict many novel ones. Finally, when training the SVM on human RBPs but searching the proteome of bacterial pathogens, we identify potential RNA-binders among secreted bacterial effector proteins which resemble host RBPs.
Based on these results, we will discuss the potential of short amino acid oligomers as a characteristic determinant for identification of specific protein groups such as RBPs and our progress on the analysis of predicted bacterial RNA-binders that contain tripeptide combinations similar to cytosolic host RBPs and thus could bind cross-species to mRNA in the host cell during infection.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

546/GRP

The biological relevance of virulence gene-associated antisense RNAs in enterohemorrhagic *Escherichia coli* O104:H4

P. Berger*[1], N. Haarmann[1], K. Förstner[2,3], J. Vogel[3], H. Karch[1], A. Mellmann[1]
[1]*University of Münster, Institute of Hygiene, Münster, Germany*
[2]*University of Würzburg, Core Unit Systems Medicine, Würzburg, Germany*
[3]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*

Introduction: Enterohemorrhagic *Escherichia coli* O104:H4 (EHEC O104:H4) caused in 2011 the largest EHEC outbreak in German history, which is also recorded as the highest incidence of EHEC-related hemolytic uremic syndrome worldwide. We recently subjected the highly pathogenic EHEC O104:H4 to differential RNA-seq (dRNA-seq), a powerful method for mapping of transcription start sites and detecting non-coding RNAs in bacteria, and performed a detailed analysis of the primary transcriptome of known virulence genes. Here, we aim to further characterize the biological relevance of the identified antisense RNA (asRNA) candidates to virulence gene regulation in EHEC O104:H4.
Methods: dRNA-seq was performed with the clinical EHEC O104:H4 isolate LB226692 grown under standard laboratory conditions (LB medium, 37C) to an OD600= 0,5. Sequencing data was processed with READemption and visualized with Integrated Genome Browser. Conservation of asRNAs in other enteroaggregative *E. coli* (EAEC) or EHEC species was tested using 5`-RACE. Expression levels of asRNAs and their target genes were determined by RT-PCR. Virulence proteins were analyzed with semi-quantitative western blot.
Results: The majority of chromosome- and pAA plasmid-encoded virulence genes were found to be associated with at least one asRNA candidate. Moreover, the asRNAs against the colonization factor dispersin and against the secreted serine proteases Pic and SigA were found conserved in other EAEC strains. The expression of the asRNAs and their targets was monitored throughout the growth phase and under different conditions. Interestingly, the asRNA against dispersin was found at least two times higher transcribed than the sense transcripts in all analyzed growth phases at 30 oC. Moreover, the higher ratio of antisense to sense transcript at 30 oC in comparison to 37 oC could be correlated with a complete abolishment of dispersin production as detected by western blot analysis.
Conclusions: Our findings suggest that at least some of the newly detected asRNAs could participate in virulence gene regulation of EHEC O104:H4. Ongoing overexpression/deletion studies will further elucidate their actual contribution and mode of action.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

547/GRP

Characterization of *Salmonella* small RNA PinT using MAPS

S. C. Santos*[1], J. Vogel[1]
[1]*University of Wuerzburg, RNA Biology Group, Institute for Molecular Infection Biology, Wuerzburg, Germany*

Small RNAs (sRNAs) are key mediators of bacterial gene expression, through post-transcriptional regulation. sRNAs regulate the expression of target genes through short imperfect base-pairing interactions, by either promoting or inhibiting transcription or by regulating the transcripts stability and degradation. Over the last decades, bacterial sRNAs have been recognized has major class of regulatory molecules involved in infection. *Salmonella* Typhimurium has 280 sRNAs, some of which have been implicated in in virulence, while many remain to be studied. Many of *Salmonella's* sRNAs are highly upregulated during infection of mammalian cells. Dual RNA-seq has been previously applied in *Salmonella* infection models, allowing the simultaneous sequencing of both host and pathogen transcriptomes. This approach has identified a previously uncharacterized sRNA, PinT. PinT is an 80 nucleotide long sRNA conserved among the *Salmonella* genus, and is up-regulated more than 100-fold during infection *Salmonella* Thyphimurium after invasion. PinT regulates the timing of the SPI-1 and SPI-2 virulence programs. Even though some of its target are known, we still lack understanding on the role of PinT and its interacting network during infection. Computational prediction of targets can be difficult and unreliable. To overcome this, we have adapted MAPS (MS2 affinity purification followed by RNA-sequencing) to identify new targets of PinT in infection-relevant conditions. Using this approach we were able to select a group of PinT candidate targets, potentially involved in infection. One of the most promising being secreted effector kinase (*SteC*) mRNA. SteC is a SPI2 effector protein that has been shown to promote bacterial survival in host tissues. With this work we aim to complement the knowledge on the biological function and regulatory network of PinT and contribute to a better understanding *Salmonella* infection.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

548/GRP

CLIP-seq laid the foundation for the molecular characterization of the virulence related proteins, CspC and CspE, in *Salmonella*

C. Charlotte [1], G. Matera*[1], E. Holmqvist[1], L. Barquist[1], T. Bischler[2], M. Sharan[1], J. Vogel[1]
[1]*University of Würzburg, Institute of Molecular Infection Biology, Würzburg, Germany*
[2]*University of Würzburg, Core Unit Systems Medicine, Würzburg, Germany*

Cold shock proteins (Csps) are defined by the presence of two RNA-binding domains and constitute one of the most conserved protein families in all three kingdoms of life. Bacterial species encode multiple Csps, several of which are constitutively expressed and whose biological function remains poorly understood. For example, *Salmonella* CspC and CspE are highly expressed during growth at 37°C and in infection-relevant conditions. To understand their physiological roles, we have used *in vivo* RNA-protein crosslinking followed by deep sequencing (CLIP-seq) [1] which provided a high-resolution map of their binding sites. Furthermore, *in silico* alignment of the common

CLIP peaks highlighted a consensus motif, CUG, as a common feature in the CspC and CspE binding regions.
To obtain a detailed molecular mechanistic view of CspC and CspE function, we used *ecnB* mRNA as a model transcript. It contains two CLIP-predicted common binding sites and it is negatively regulated in the double *cspCE* deletion strain. We found that the expression of the *ecnB* mRNA depends on the presence of CspC or CspE, which bind and stabilize the transcript post-transcriptionally. To dissect the molecular determinants of Csp-mediated regulation of the *ecnB* mRNA, we used *in vitro* gel shift assays with different mutants of the predicted binding sites. Only one of the two CLIP peaks of *ecnB* is essential for binding of CspC and CspE. Moreover, a single point mutation in the CUG motif inside this peak decreased significantly the affinity to the proteins, validating the *in silico* prediction. Computation of *ecnB* mRNA folding revealed that the second peak is involved in the formation of a stem-loop, suggesting that both sequence and structural elements could indeed be required for binding to CspC and CspE.

References
[1] Holmqvist, E., Wright, P.R., Li, L., Bischler, T., Barquist, L., Reinhardt, R., Backofen, R., Vogel, J., 2016. Global RNA recognition patterns of post-transcriptional regulators Hfq and CsrA revealed by UV crosslinking in vivo. EMBO J. 35, 991–1011. doi:10.15252/embj.201593360

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
General and Hospital Hygiene (StAG HY)

549/HYP
Understanding the molecular mechanisms involved in the spore inactivation by plasma sterilization
M. Raguse[1], M. Fiebrandt[2], K. Stapelmann[2], P. Awakowicz[2], P. Eaton[3], P. Eichenberger[4], T. Douki[5], R. Moeller*[1]
[1]*German Aerospace Center , Space Microbiology Research Group, Cologne, Germany*
[2]*Ruhr-Universität Bochum , Lehrstuhl für Allgemeine Elektrotechnik und Plasmatechnik, Bochum, Germany*
[3]*University of Porto, Faculty of Sciences, Porto, Portugal*
[4]*New York University, Center of Genomics and System Biology, New York, United States*
[5]*CEA-Grenoble, Service de Chimie Inorganique et Biologique, Grenoble, France*

Being the most resistant form of a biological system, spores of *Bacillus subtilis* are very resistant against a broad spectrum of sterilization methods and, therefore, are commonly used as a biological indicator in order to verify the functionality of a sterilization procedure. The process of low-pressure plasma sterilization is a promising alternative to conventional sterilization methods as it is extremely fast, efficient and gentle to heat-sensitive materials. Active plasma species contain a high degree of sporicidal UV/VUV-radiation, as well as charged particles and free radicals, which exert detrimental effects on microorganisms. In this study we present novel insights into the key factors involved in spore inactivation by low pressure plasma sterilization using a double inductively-coupled plasma reactor.
In order to standardize the assessment of spore inactivation efficiencies by plasma discharges, an electrically operated deposition device was developed, allowing fast, reproducible, and homogeneous preparation of *B. subtilis* spore in monolayers on surfaces leading to more reliable investigations. We demonstrate that low-pressure plasma discharges of argon and oxygen discharges cause significant physical damage to spore surface structures as visualized by atomic force microscopy. A systematic analysis of *B. subtilis* spores lacking individual coat and crust layers - the first barrier to environmental influences – revealed the coat to be a major factor in spore resistance towards plasma treatment (Raguse et al., 2016).
In order to gain a better understanding of the complex molecular mechanisms involved in the inactivation by plasma sterilization processes, we analyzed plasma-induced DNA lesions in vitro, identified general and spore-specific DNA lesions, and characterized different DNA repair mechanisms during spore revival after plasma treatment.

References
[1] Raguse M, Fiebrandt M, Stapelmann K, Madela K, Laue M, Lackmann JW, Thwaite JE, Setlow P, Awakowicz P, Moeller R. Improvement of Biological Indicators by Uniformly Distributing *Bacillus subtilis* Spores in Monolayers To Evaluate Enhanced Spore Decontamination Technologies. Appl Environ Microbiol. 2016 Jan 22;82(7):2031-8. doi: 10.1128/AEM.03934-15.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

550/HYP
Development of a site-specific bioluminescence-based test system for *in vivo* evaluation of antimicrobial coatings
M. Czieborowski*[1], L. Kauling[1], B. Philipp[1]
[1]*University of Münster, Institute of Molecular Microbiology and Biotechnology, Münster, Germany*

In almost every technical system, which is in contact with aqueous liquids, bacterial biofilm formation occurs and may cause hygienic problems. Antimicrobial coatings of surfaces are a feasible approach for minimizing biofilm formation and, thus, for increasing the safety and performance in such technical installations.
For establishing novel antimicrobial coatings it is necessary to evaluate the efficiency of the new material. The Japanese Industrial Standard JIS Z 2801/ ISO 22196 assay is routinely used for this purpose. However, this test does not reflect the biotic and abiotic conditions prevailing in many technical water installations, e.g. in drinking water filtration where the concentration of organic nutrients is extremely low.
Thus, the goal of this study is to develop a site-specific test system for antimicrobial coatings for ultrafiltration membranes, which is based on bacteria that actually occur in the respective sites of application. As bioluminescence is a well-established indicator of bacterial fitness and allows *in vivo* analysis of antibacterial effects, the respective test bacteria will be equipped with the *lux*-operon.
Preliminary work with a bioluminescent strain of *Escherichia coli* MG1655 showed that bioluminescence can be detected inside the fibers of ultrafiltration membranes and that the respective antimicrobial compounds designated for the use in the coatings interfere with the cells ability to perform bioluminescence. In the next step, bacteria were isolated from commercial membranes for drinking water ultrafiltration and identified as *Alphaproteobacteria* of the genera *Sphingomonas* and *Novosphingobium*. These genera are known to occur in anthropogenic as well as in oligotrophic natural environments. The isolated bacterial strains are currently being characterized regarding substrate spectra, biofilm formation as well as resistance and survival of stress conditions, especially starvation. Selected strains will then be engineered as bioluminescent reporter strains and used for in vivo analysis of biofilm formation in coated and non-coated membrane fibers.
The approach pursued in this study has the potential to evaluate the performance of antimicrobial coatings with site-specific bacterial strains under conditions close to the *in-situ* situation.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

551/HYP

Outbreak - The management game for fighting infectious diseases

J. Rasch*[1], S. Böhme[1], N. Janßen[1], K. Schaab[1], A. Klages[1], S. Pulst[1], C. Herrmann[1], M. Steinert[1]
[1]*TU Braunschweig, Mikrobiologie, Braunschweig, Germany*

Infectious diseases are one of the most frequent contributors to human morbidity and mortality, causing extensive public health issues. It was a big mistake to think that infectious diseases are regressive. Actually, since the last two decades there are new epidemics and old ones experience a renaissance. At the same time antibiotics are losing their efficiency, because pathogens develop resistances. Worldwide mobility, climate change, aging of the population and new pathogens and variants will aggravate the situation more and more. This makes the development of new strategies for health management to an important task for the future.

Outbreak is a computer based management game, in which the players are asked to find new strategies to prevent and fight infectious diseases in the world. Therefore, strategies are developed and operated in the fields research, prevention management, outbreak management and surveillance. A successful health management is measured in three categories: morbidity and mortality, economic wealth of the population and acceptance of the chosen methods. While playing this game, the players learn to transfer and exercise their knowledge of infectious diseases in a realistic scenario. Thereby, a holistic view on the topic is generated in an attractive teach-study-environment. The players get sensitized for the present challenges of global infectious diseases and learn to use the relevant scientific methods and approaches. Finally, the players learn to act responsible and to see a holistic context.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

552/HYP

Evaluation of Fourier Transform Infrared (FTIR) Spectroscopy for Typing of Clinical *Klebsiella pneumoniae* Isolates

A. Dinkelacker*[1], J. Rau[2], N. Mauder[3], J. Liese[1]
[1]*University Hospital Tübingen, Tübingen, Germany*
[2]*Chemisches und Veterinäruntersuchungsamt Stuttgart, Fellbach, Germany*
[3]*Bruker Daltonik GmbH, Bremen, Germany*

Background: *Klebsiella (K.) pneumoniae* is a clinically relevant pathogen, which can cause a wide range of infections as well as nosocomial outbreaks. During outbreak management, quick and reliable strain typing is desirable to detect transmission events or environmental reservoirs, but commonly used DNA-based typing methods for *K. pneumoniae* are costly and/or labor intensive. Fourier transform infrared (FTIR) spectroscopy is a technique, which gathers information about the chemical composition of the bacterial isolates and which has been used successfully for species identification and strain typing, especially in the context of food-borne or veterinary pathogens.

Objectives: The aim of this study was to evaluate FTIR spectroscopy as a tool for typing of clinical *K. pneumoniae* isolates in comparison to a commercial rep-PCR typing system.

Materials and methods: A set of 38 *K. pneumoniae* strains was collected during a suspected *K. pneumoniae* outbreak on a neonatal intensive care unit. All isolates were analyzed with a commercial rep-PCR system (Diversilab®, BioMérieux) according to the manufacturers instructions. For FTIR spectroscopy analysis *K. pneumoniae* isolates were cultivated for 24 h ± 30 min on sheep blood agar at 37°C. Colonies were then suspended in de-ionized water and spotted in duplicates on a zinc selenide optical plate. The dried film was used directly for FTIR spectroscopy. Analysis was performed on a Tensor 27 spectrometer with an HTS-XT module (BrukerOptics). Results were analyzed with the OPUS software package (BrukerOptics), RapidMiner and PAST.

Results: The 38 *K. pneumoniae* isolates were grouped into 4 clusters (comprising 6-13 strains) and 4 singletons by rep-PCR, when a cutoff of 90% pattern identity was chosen. The phylogeny obtained from FTIR spectra showed 4 clusters (6-12 isolates per cluster) and 3 singletons. 35 of 38 isolates (92%) were assigned to the same cluster by FTIR analysis when compared to rep-PCR. Processing time was around 11 hours for rep-PCR, whereas processing time for FTIR spectroscopy was about 3 hours.

Conclusions: In our setting, FTIR spectroscopy results showed high accordance in comparison to rep-PCR for typing *K. pneumoniae* isolates from routine cultures. This technique has the potential to be used routinely in clinical settings due to fast turnaround times and low costs per sample. Larger studies with a broader spectrum of clinically relevant species and comparison with whole genome sequencing analysis will be helpful to further determine the value of FTIR spectroscopy for routine surveillance and rapid outbreak analysis.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

553/HYP

Assessment of compatibility of microbial barrier properties of packaging material of terminally sterilized products with the airborne microbial challenge during the storage period

H. Dunkelberg*[1]
[1]*Labor Dunkelberg, Bad Sooden-Allendorf, Germany*

Question: The question of this presentation was to demonstrate the compatibility of barrier performance (i.e. filtration efficiency) of medical packaging material with the exposure to airborne microorganisms during the storage period.

Methods: The compatibility of the microbial filtration efficiency of the packaging material with the airborne microbial challenge (N_0) was calculated according to this equation:

N_0 x [100-filtration efficiency (%)]/100 x n =< 10^{-6}

where

N_0 = airborne microbial challenge; it is depends of the airborne microbial concentration and the volume of air that enters the packaging during storage (caused by air pressure and temperature variations, see the Boyle-Mariotte and Gay-Lussac laws),

n = number of events causing a microbial challenge according to N_0,

10^{-6} = sterility assurance level (SAL).

The exposure chamber method was used to detect the filtration efficiency of paper/film pouches (16x18 cm) denoted by A and B and of non-woven/film pouches denoted by C. 30 pouches per group were charged with uncovered thermo-resistant and CASO-agar filled petri dishes, sealed and sterilized, and then exposed. The exposure chamber with a capacity of 1 m³ was equipped with a vacuum pump in order to reduce the atmospheric pressure periodically by 70 hPa which leads to an air flow through the permeable component of the packaging. A microbial aerosol of Micrococcus luteus with a mean particle size of about 3 µm was generated by a nebulizer. The pressure in the chamber was measured digitally with a pressure sensor and a data logger. A glass impinger air sampler was used to determine the airborne microbial concentration. After exposure, the pouches were incubated at 36 °C for 72 hours. The number of colony forming units (CFU) was registered after removing the packaging material. The filtration efficiency was calculated by means of the ratio of the microbial challenge and the CFUs observed on the dishes.

The following example was used to calculate the maintenance of sterility. Volume of the pouch: 150 cm³, one event reflects a daily temperature variation of 2 °C at room temperature of 20 °C, and an airborne microbial concentration of 10 CFU (particle size: < 3 µm).
Results: The filtration efficiencies of the pouches type A were 98.4, of type B 98.8 and of type C 85.6 % respectively. The air volume that enters the packaging per one event according to the given example was about 1 cm³, a microbial challenge of 0.00001 microbes could be calculated for one event. We obtained the following results for n (= number of events or days) which demonstrate that the used packaging material maintains sterility at the SAL: n= 6.25 (type A), 8.3 (type B) and 0.7 (type C).
Conclusion: The filtration efficiency and the estimated airborne microbial challenge were used to assess the compatibility of the medical packaging material with specified exposure conditions at the SAL. The tested pouches guaranteed sterility at the SAL for only a few days.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Membranes and Transport (FG MT)

554/MTP
Interplay of gold and copper resistance in *Cupriavidus metallidurans*
L. Bauer*[1], N. Wiesemann[1], M. Herzberg[1], M. Altzschner[2], A. Holleitner[2], G. Hause[3], L. Berthold[4], D. Dobritzsch[5], S. Baginsky[5], F. Reith[6], D. H. Nies[1]
[1]*Martin-Luther University Halle-Wittenberg, Molecular Microbiology, Halle, Germany*
[2]*TU Munich, Walter Schottky Institut and Physik-Department, Garching, Germany*
[3]*Martin-Luther University Halle-Wittenberg, Microscopy, Halle, Germany*
[4]*Frauenhofer Institute for Microstructure of Materials and Systems, Halle, Germany*
[5]*Martin-Luther University Halle-Wittenberg, Plant Biochemistry, Halle, Germany*
[6]*The University of Adelaide, School of Biological Sciences, The Sprigg Geobiology Centre, Centre of Tectonics, Resources and Exploration (TRaX), Adelaide, Australia*

Cupriavidus metallidurans lives in environments containing high concentrations of transition metals cations. Australian soils overlying gold (Au)-deposits contain a mixture of these plus toxic concentration of Au-complexes. Resistance determinants on the two native plasmids of *C. metallidurans* are strongly up-regulated in the presence of transition metal mixtures characteristically for these auriferous soils, however, they not involved in Au resistance. The most important of these plasmid-encoded resistance factors are metal efflux pumps and their function is to prevent cellular accumulation of the transition metals (Ni, Co, Zn, Cu) that usually accompany toxic Au-complexes in these environments. While most of the transition metal cations of the first transition period do not interfere with Au-resistance, Cu(II) and Au-complexes strongly influence each other"s toxicity in a negative cooperative manner. The two most important contributors to this interplay were characterized using a combination of biochemical, microscopic and physiological methods. One is a Cu-resistance factor that is inhibited by Au-complexes, the other is a Cu-dependent enzyme that controls the formation of Au-nanoparticles in *C. metallidurans*. Thus, the interplay of copper and gold resistance pathways in *C. metallidurans* is understood from the molecular to the ecological level.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

555/MTP
Phosphotransferase systems (PTS) form distinct cluster in the membrane of Corynebacterium glutamicum
G. Benevides Martins*[1], M. Bramkamp[1]
[1]*Ludwig-Maximilians Universität München, Department Biologie I, Ber. Mikrobiologie, München, Germany*

Corynebacterium glutamicum grows on a variety of carbohydrates that are in part taken up and phosphorylated by membrane-integral sugar specific phosphotransferase systems (PTS). Because *C. glutamicum* is widely used as producer of glutamate and amino acids in industry, information regarding sugar uptake systems and membrane economy are useful to understand specific sugar uptake rates and their underlying molecular mechanisms.
Here, we analyzed localization of the fructose specific PtsF and the glucose specific PtsG transporters. To this end, the transmembrane-integral components of the phosphotransferase systems were tagged with fluorescent reporters. To avoid overexpression and artificial expression heterogeneity, fusion constructs were inserted as allelic replacement in the genome of wild type *C. glutamicum* (RES 167) cells. The strains were grown in CGXII minimal medium containing two different carbon sources: the specific sugars taken up by each Pts (fructose and glucose), acetate and mixtures of both. Subcellular localization of the Pts proteins was subsequently analyzed by fluorescence microscopy.
PtsG and PtsF form membrane embedded clusters that localize in a punctate pattern within the cell membrane. The size of the observed clusters changes upon presence or absence of the transported substrate. In presence of the correct Pts sugars the clusters significantly increase in size, supporting the notion that transport complexes associate under these conditions. We next addressed the influence of surface area to volume ratios on the Pts localization. Therefore, L-forms were generated by culturing the cells in MSM/NB osmo-protective medium, in the presence of D-cycloserine. D-cycloserine inhibits the alanine racemase (Alr) and the D-alanine:D-alanine ligase (Ddl), thereby effectively inhibiting cell wall synthesis. Although L-forms have larger membrane surfaces PtsG/F foci were similar compared to that of rod-shaped cells. This suggests that the cluster size of Pts proteins is intrinsically regulated and not a consequence of membrane space.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

556/MTP
Molecular basis of membrane potential-generating system in *Staphyloccous aureus*
S. H. Fan*[1], S. Reichert[1], F. Goetz[1]
[1]*Interfaculty Institute for Microbiology and Infection Medicine Tübingen (IMIT), Microbial Genetics, Tuebingen, Germany*

In many aerobic or facultative microorganisms, the major electron donor is $NADH+H^+$ produced for example during glycolysis and the TCA cycle. In *Escherichia coli,* this is accomplished by NADH:quinone oxidoreductase (Ndh1). Recently, it has been shown in our lab that *Staphylococcus aureus* does not possess such a Ndh1 complex, but a non-electrogenic type 2 (Ndh2) protein that oxidizes $NADH+H^+$. In addition, a protein called MpsA (*mps* for membrane potential-generating system) was identified in *S. aureus* showing sequence similarity to the proton-translocating subunit NuoL of complex 1 in *E. coli*. *mpsA* is the first part of an operon comprising three genes: *mpsA, mpsB and mpsC*. MpsB and MpsC show no significant sequence homologies to proteins with known function. However, we have found that MpsB carries a conserved metal binding motif representing a

domain that is found in carbonic anhydrases. Carbonic anhydrase (CA) catalyzes the interconversion of $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$. The equilibrium of these forms is important for proper physiological functioning of the cells. We assume that MpsB in *S. aureus* might play a role in CO_2 transport and respiration. Therefore, this study aims to investigate the involvement of MpsB in respiration, especially in CO_2 and/or bicarbonate metabolism. Deletion mutant of the *mpsB* and its complementation vector were constructed. Preliminary experiments were performed to characterize its growth on solid and liquid medium in aerobic and high (5%) CO_2. Under normal conditions the growth of Δ*mpsB* was severely affected; however, in the presence CO_2 it was almost like the wild type. The pH profile of the mutant was also altered compared to the wild type. With regard to these observations, MpsB in *S. aureus* is postulated to be an integral part in CO_2 metabolism and energy conservation.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

557/MTP

Biological role of outer membrane vesicle (OMV)-associated small proteins derived from the phytopathogen *Agrobacterium tumefaciens*

L. R. Knoke*[1], K. Götz[1], S. Schäkermann[2], J. Bandow[2], M. Aktas[1]
[1]*Ruhr University Bochum, Department of Microbial Biology, Bochum, Germany*
[2]*Ruhr University Bochum, Applied Microbiology, Bochum, Germany*

Bacterial extracellular membrane vesicles are considered as novel players in various cell communication systems. Outer membrane vesicles (OMVs) are released from the outer membrane of many Gram-negative bacteria. These extracellular nanostructures comprise distinct biomolecules such as small compounds, DNA, RNA, proteins and peptides, which are involved in intra- and inter-species communication and pathogenicity (Kulp and Kuehn 2010; Schwechheimer and Kuehn, 2015).
We have recently demonstrated that the phytopathogen *Agrobacterium tumefaciens*, the causative agent of crown gall disease, releases OMVs into the culture supernatant during growth. A proteome study of the OMV fractions obtained under different growth conditions identified a total of about 60 proteins including the two conserved small proteins, Atu8019 (53 aa) and Atu2614 (83 aa) of unknown function. Both proteins contain a putative signal peptidase II cleavage site and might represent membrane lipoproteins. Atu8019 shares sequence similarities to entericidin antidote/toxin peptides whereas Atu2614 contains a highly hydrophobic C-terminal domain with several extended glycine zipper motifs, which are commonly found in pore forming membrane proteins. To elucidate the biological role of these two small proteins we combine biochemical and mutagenesis approaches. We expressed recombinant Atu8019-His in *A. tumefaciens* and confirmed that it is highly associated with OMVs. We have been successful to purify sufficient amounts of recombinant Atu8019-His to homogeneity from *A. tumefaciens* membrane fractions which allows us in the near future to biochemically characterize Atu8019.

References

[1] Kulp A, Kuehn MJ. 2010. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu Rev Microbiol. 64: 163–184.
[2] Schwechheimer C, Kuehn MJ. 2015. Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions. Nat Rev Microbiol. 13:605-619.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

558/MTP

Establishment of monoclonal antibodies against capsule polymerases of *Escherichia coli* K1 and *Neisseria meningitidis* serogroups C and Y

S. Manig*[1], H. Claus[1], U. Vogel[1]
[1]*Institute of Hygiene and Microbiology, Wuerzburg, Germany*

Introduction: *Neisseria meningitidis* and *Escherichia coli* K1 cause bacterial meningitis and sepsis. A major virulence factor of *N.meningitidis* serogroups A, B, C, W, X and Y and *E.coli* serogroup K1 is the sialic acid containing capsular polysaccharide. Monoclonal antibodies directed against polysialyltransferases (cp) NeuS, Csb, Csc, Csw and Csy are not available.
Objectives: Monoclonal antibodies against the capsule polymerases NeuS, Csc and Csy are generated, which can be used to study molecular interactions of capsule synthesis, modification and transport proteins.
Methods: Monoclonal antibodies were generated using standard protocols (Köhler *et al,* 1975). Mice were immunized with recombinant proteins of NeuS, Csc and Csy. ELISA, Western blot, and immunofluorescence were used for screening.
Results: At the time of writing, cells producing monoclonal antibodies of Csc, Csy and NeuS were cloned twice. Three lines of Csy-, three of Csc-, and four of NeuS-hybridomas were followed. There were three, 8 and 16 lines specifically reactive with the immunizing protein for Csy, Csc, and NeuS, respectively.
Conclusion: We report on the ongoing establishment of monoclonal antibodies reactive against Csy, Csc, and NeuS. We expect to present hybridoma clones with specific reactivity. Cell lines will be made available to the community as soon as possible.

References

[1] Köhler G, Milstein C. (1975): Continuous cultures of fused cells secreting antibody of predefined specificity.*Nature* 256, 495–497.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

559/MTP

Key players of a type IV secretion system mediating antibiotic resistance spread among enterococci and staphylococci

I. Probst*[1,2], V. Kohler[3], C. Faller[2], C. Fercher[3], N. Goessweiner-Mohr[4,5], G. Koraimann[3], W. Keller[3], E. Grohmann[1,6]
[1]*University Medical Centre Freiburg, Division of Infectious Diseases, Freiburg, Germany*
[2]*Albert-Ludwigs-University Freiburg, Institute of Biology II, Microbiology, Freiburg, Germany*
[3]*Karl-Franzens-University Graz, Institute for Molecular Biosciences, Graz, Austria*
[4]*University Medical Centre Hamburg-Eppendorf (UKE), Centre for Structural Systems Biology (CSSB), Hamburg, Germany*
[5]*Institute of Molecular Biotechnology (IMBA), Austrian Academy of Sciences, Vienna, Austria*
[6]*Beuth University of Applied Sciences Berlin, Department of Life Sciences and Technology, Berlin, Germany*

Discovery void of novel types of antibiotics and increasing antibiotic resistances in pathogenic bacteria present a serious health issue worldwide. Conjugative DNA transfer is the most important transmission path for antibiotic resistance and virulence gene dissemination among bacteria and is mediated by a protein complex, generally known as type IV secretion system (T4SS). Enterococci are the third most common cause of healthcare-associated infections, which include urinary tract infections, bacteremia and endocarditis. A conjugative model system in Gram-positive bacteria is the T4SS from broad-host-range plasmid pIP501, frequently encountered in nosocomial pathogens, such as *Enterococcus faecalis* and *Enterococcus faecium*. Postulated key factors of the conjugative transfer complex are the relaxase TraA, two ATPases, TraE and TraI/TraJ, the first

putative two-protein T4S coupling protein, the muramidase TraG, the putative channel factors, TraF, TraK, TraL and TraM and the surface factor TraO[1]. To elucidate the role of the pIP501 Tra proteins we generated a number of single marker-less *tra* knock-out mutants in *E. faecalis* harbouring pIP501 by using a method consisting of two homologous recombination steps. Till now we have generated the deletion mutants, *E. faecalis* pIP501Δ*traE*, Δ*traF*, Δ*traG*, Δ*traH*, Δ*traI*, Δ*traJ*, Δ*traK*, Δ*traL,* Δ*traM* and Δ*traN*. Biparental matings showed that TraE, TraF, TraG, TraH, TraI, TraJ, TraK, TraL and TraM are essential for pIP501 conjugative transfer. *In trans* complementation of the respective deleted gene excluded possible polar effects on downstream genes in the *tra* operon. We also demonstrated that the peptidoglycan-degrading domain SLT of TraG is not sufficient to complement the *E. faecalis* JH2-2 pIP501Δ*traG* mutant[2]. TraN has been shown to be a DNA binding protein, binding sequence-specifically upstream of the pIP501 *oriT nic* site[3]. It acts as a negative regulator of pIP501 transfer, presumably by either interfering with TraA relaxase activity or by negatively regulating *tra* operon expression. Generation of the knock-outs *traB*, *traC*, *traD* and *traO* is in progress and will help decipher the molecular mechanism of the pIP501 conjugative transfer machinery. Here we will present the current state of knowledge of the pIP501 T4S model system.

References

[1] Fercher C, Probst I, Kohler V, Goessweiner-Mohr N, Arends K, Grohmann E, Zangger K, Meyer NH and Keller W (2016). VirB8-like protein TraH is crucial for DNA transfer in *Enterococcus faecalis*. Scientific Reports 6:24643, doi:10.1038/srep24643.
[2] Kohler V et al., manuscript in preparation.
[3] Goessweiner-Mohr N, Eder M, Hofer G, Fercher C, Arends K, Birner-Gruenberger R, Grohmann E and Keller W (2014) Structure of the double stranded DNA binding type IV secretion protein TraN from Enterococcus. Acta Cryst Section D, Biological Crystallography, 70, 2376-2389.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

560/MTP

Functional analyses of the AAA-ATPases PilT1 and PilT2 of *Thermus thermophilus*

K. Kruse*[1], T. Schlemmer[1], B. Averhoff[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt a. M., Germany*

Introduction: Bacterial DNA transfer is a natural and powerful means for single cell organisms to communicate information that are beneficial to compete and survive in shared biotopes. To get insights into the structure and function of DNA translocators we chose the thermophilic bacterium *Thermus thermophilus* HB27 and identified 16 proteins being essential for natural transformation[1]. Several proteins, including the pilus assembly ATPase PilF, were found to play a dual role in natural transformation and biogenesis of type IV pili (T4P)[2,3]. Recently we identified two retraction ATPases, PilT1 and PilT2[3]. Both are essential for T4P disassembly but dispensable for natural transformation.
Objectives: Next, we aimed to get insights into the structure and function of the retraction ATPases PilT1 and PilT2.
Materials & Methods: To enable a biochemical characterization of both PilT proteins we performed heterologous production of PilT1 and PilT2 in *E. coli* followed by purification via affinity chromatography and detection of the ATPases by Western blot analyses.
Results: Both *T. thermophilus* retraction ATPases were produced in *E. coli* and purified by heat precipitation and affinity chromatography. PilT1 and PilT2 hydrolysed ATP with an activity of ~30 nmol Pi×min^{-1}×mg protein^{-1} and ~80 nmol Pi×min^{-1}×mg protein^{-1}, respectively. ATPase activities were detected over a pH range from 6.5 to 10.5 with maximal activity of PilT1 at pH 6.5 and PilT2 at pH 9.5. PilT1 and PilT2 both formed complexes with a molecular mass of ~260 kDa, indicating that the 42 kDa PilT1 and the 43 kDa PilT2 monomers form hexamers.
Conclusion: PilT1 and PilT2 both exhibit ATPase activity and form hexameric complexes.

References

[1] Averhoff, B. (2009) Shuffling genes around in hot environments: the unique DNA transporter of *Thermus thermophilus*. *FEMS Microbiol Rev,* 33: 611-626.
[2] Rose, I., Biuković, G., Aderhold, P., Müller, V., Grüber, G. and Averhoff, B. (2010) Identification of a unique, zinc-containing transport ATPase essential for natural trasformation in *Thermus thermophilus* HB27. *Extremophiles,* 15: 191-202.
[3] Salzer, R., Joos, F. and Averhoff, B. (2014) Type IV pilus biogenesis, twitching motility, and DNA uptake in *Thermus thermophilus*: Discrete roles of antagonistic ATPases PilF, PilT1 and PilT2. *Appl Environ Microbiol,* 80: 644-652.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

561/MTP

Identification of the lipid moiety structure of Staphylococci by electrospray ionization mss spectrometry

H. Wang*[1], A. Kulik[1], P. Popella[1], F. Götz[1]
[1]*University of Tübingen, Tübingen, Germany*

Bacterial lipoproteins (Lpp) represent a major class of membrane proteins. They are distinguished by a lipid moiety at the N-terminus by which they are anchored in the outer leaflet of the cytoplasmic membrane. Particularly in Gram-positive bacteria Lpp are important agonists of the immune system via Toll-like receptor 2 (TLR2) activation. Di-acylated and tri-acylated lipoproteins activate the immune response through the heterodimer, TLR2/6 and TLR2/1, respectively. However, the structure of Staphylococcal lipoprotiens remains controversial with respect whether the lipid moiety is di- or triacylated or a mixture of both. This work employed electrospray ionization (ESI) mass spectrometry to analyse a shortened lipopeptide in *S. aureus* strain. We confirm that the *S. aureus* lipopeptide is triacylated under certain environment conditions, which provides evidence for the existence of the unknown N-acylation enzyme. It is in the hope that this work would facilitate the revelation of the TLR2 receptor recognition.

References

[1] Kurokawa, K., M.S. Kim, R. Ichikawa, K.H. Ryu, N. Dohmae, H. Nakayama & B.L. Lee, (2012) Environment-mediated accumulation of diacyl lipoproteins over their triacyl counterparts in Staphylococcus aureus. *Journal of bacteriology* 194: 3299-3306.
[2] Nakanishi T., Shimizu A., (2000) Determination of ionization efficiency of glycated and non-glycated peptides from the N-terminal of hemoglobin β-chain by electrospray ionization mass spectrometry. *Journal of Chromatography B* 746: 83–89.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

562/MTP

Aqueous access of amino acids residues of membrane-integral AtpI in *Escherichia coli* and *Acetobacterium woodii*

D. Klütsch*[1], G. Deckers-Hebestreit[1], D. Wolbers[1], C. Hübert[1]
[1]*Universität Osnabrück, Osnabrück, Germany*

The *atpI* gene is present in most bacteria as the first gene of the *atp* operon, but the amino acid sequences show a high variability. In *E. coli* it codes for AtpI, a hydrophobic protein of 14 kDa. H^+-translocating F_OF_1-ATP synthases do not absolutely require AtpI for its assembly, but the stability and activity of the membrane-bound enzyme complex is modestly increased in the presence of AtpI (Deckers-Hebestreit, 2013, and references therein). However, AtpI is essential for Na^+-pumping F_OF_1-ATP synthases like in the anaerobic bacterium *A. woodii*. In this case deletion of *atpI* results in mis-assembly caused by loss of a stable oligomerization of the *c* ring. Whereas Na^+-F_OF_1-ATP synthase of *A. woodii* heterologously expressed in *E. coli* is functional in the presence of the native AtpI (Brandt et al, 2013), it cannot be substituted by AtpI of *E. coli*.

AtpI has been predicted to have four transmembrane helices with a N_{in}-C_{in} orientation. The topology of AtpI was verified using intact cells as well as inverted membrane vesicles for labelling of single cysteine-substituted variants with thiol-reactive maleimides. After treatment with membrane-impermeable 4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid to block thiol groups accessible from the water phase and the subsequent incubation with membrane-permeable 3-(N-maleimido-propionyl) biocytin, AtpI proteins were purified by affinity chromatography via a His-tag fused to the C-terminus and detected using fluorescently labelled streptavidin as well as anti-His antibodies for western blot analyses (Jung et al, 1998).

Our analysis verifies the presence of four transmembrane regions in *E. coli* and *A. woodii*. Studies on the impact of the cysteine-substituted *A. woodii* AtpI variants on the assembly of the *c* ring are in progress. Only a few amino acid residues were accessible from the water phase at the periplasmic side of the membrane. However, the accessibility of both termini as well as the cytoplasmic loop is highly extended. Short membrane-spanning segments indicate that the helices may form a water-accessible cavity in the membrane. A hydrophilic cavity has also been described for F_O-*a* in the Na^+-pumping F_OF_1-ATP synthase of *Propionigenium modestum* (von Ballmoos et al, 2002) or the H^+-pumping F_OF_1-ATP synthase of *Polytomella sp*. mitochondria (Allegretti et al, 2015). Therefore, comparable accessibility studies on the membrane-spanning segments of E. coli F_O-*a* are now under investigation. We hypothesize that AtpI forms a four-helix bundle comparable to F_O-*a* essential for the oligomerization of the *c* ring.

References

[1] Allegretti et al (2015) *Nature* 521, 237-240.
[2] Brandt et al (2013*) J Bioenerg Biomembr* 45,15-23.
[3] Deckers-Hebestreit (2013) *Biochem Soc Trans* 45, 1288-1293.
[4] Jung et al (1998) *J Biol Chem* 273, 26400-26407.
[5] von Ballmoos et al (2002) *Eur J Biochem* 269, 5581-5589.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

563/MTP

Characterization of the *E. coli* proteome in response to high rate Tat-mediated protein secretion

K. Dolata*[1], I. Guerrero-Montero[2], C. Robinson[2], S. Sievers[1], J. Hoyer[1], D. Becher[1], K. Riedel[1]
[1]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*
[2]*University of Kent, Centre for Molecular Processing, School of Biosciences, Canterbury, United Kingdom*

E. coli serves as an excellent model for production of recombinant proteins. Typically, secretion occurs via the general secretory pathway (Sec), which transports proteins through the plasma membrane in a reduced, unfolded state. Nevertheless, Sec secretion platform suffers from severe limitations hampering the expression of heterologous proteins. Recently, the twin-arginine translocation pathway (Tat) gained a lot of interest due to its ability to transport fully folded proteins to the periplasm[1]. Thus, it has been shown to be an interesting alternative to Sec-dependent protein secretion. Despite the high potential of Tat pathway, an overexpression of recombinant proteins invariably induces secretion stress which diminishes production efficiency[2].

In this study we investigated, the alterations of *E. coli* proteome under Tat secretion stress.

To gain insight into the production stress response we analyze the effect of a depletion of Tat components and an overproduction of Tat substrates and their misfolded derivatives on the proteome of *E. coli*. To study membrane, periplasmic and cytosolic fractions we applied SDS-PAGE and LC-MS/MS, followed by data visualization with Voronoi treemaps.

Secretion of misfolded Tat substrates resulted in an increased abundance of proteins of the oxidative-, the heat- and the cold shock stress response. Moreover, an increased periplasmic stress response was detected in the post-induction phase of mutated *versus* Tat-compatible proteins. Tat depletion, however, resulted in downregulation of stress response proteins.

The study shows that *E. coli* proteome undergoes specific alterations throughout Tat secretion. Additionally, the production of misfolded Tat substrates results in induction of stress response proteins.

References

[1] Heli I. Alanen, et al. Efficient export of human growth hormone, interferon α2b and antibody fragments to the periplasm by the Escherichia coli Tat pathway in the absence of prior disulfide bond formation, Biochimica et Biophysica Acta, December 2015, 756–763
[2] Kotzsch A., Vernet E., Hammarström M., Berthelsen J., Weigelt J., Gräslund S., Sundström M. A secretory system for bacterial production of high-profile protein targets. Protein Science, 2011 March; 20(3):597-09.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

564/MTP

The role of porins in antibiotic uptake

D. Hörömpöli*[1], A. Berscheid[1], H. Brötz-Oesterhelt[1]
[1]*Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), Microbial Bioactive Compounds, Tübingen, Germany*

The outer membrane of Gram-negative bacteria is the first layer of protection. Hydrophilic molecules cannot diffusive passively across the asymmetric lipid bilayer, which is why channels and transporters are needed for nutrient uptake. Changes in regulation of outer membrane protein expression enable bacteria to adapt to a changing environment, leading to controlled uptake of substances. One class of outer membrane proteins are porins. In *E. coli*, hydrophilic, small (<600 Da) molecules, including several antibiotics, can pass these water filled channels passively via diffusion and enter the periplasm. The general porins of *E. coli* show a high grade of sequence similarity, but varying substrate

specificities due to differences in their constriction sites (Pagès *et al.* 2008). Alterations in porin expression or the loss of a specific porin may result in an increased antibiotic resistance, as it was shown in various clinical and mutational studies.
In order to investigate the role of porins in antibiotic uptake, *E. coli* strains lacking multiple porins were generated by replacing porin genes with an antibiotic resistance cassette via homologous recombination. Here, deletion of multiple, similar porins may result in an additive effect, leading to an even higher antibiotic resistance compared to single porin knockouts. In contrast, overexpression of porins could lead to an increased uptake of antibiotics. Since porin overexpression has been previously described as toxic to the cells, various vector systems were compared as porin overexpression constructs for physiological studies in *E. coli*. The effects of altered porin expression were determined in different media by testing the minimal inhibition concentrations of several antibiotics as well as by growth curve experiments. First results indicate that the choice of plasmid can severely impact on cell viability in minimal media. The choice of the right plasmid system is therefore important for physiological studies.
A deeper understanding of the physicochemical properties of a molecule that are crucial for outer membrane passage via porins will aid future drug design.

References
[1] Pagès, J. M., James, C. E., & Winterhalter, M. (2008). The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. *Nature Reviews Microbiology*, *6*(12), 893-903.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

565/MTP
The protein component of the signal recognition particle Ffh is subject to Lon-dependent proteolysis in *Escherichia coli*
B. Sauerbrei*[1], J. Arends[1], F. Narberhaus[1]
[1]*Ruhr University Bochum, Microbial Biology, Bochum, Germany*

Transport of membrane proteins is an essential process to sustain cellular homoeostasis and membrane integrity. One mechanism for membrane protein trafficking is the cotranslational transport by the signal recognition particle (SRP) [1]. The prokaryotic SRP consists of the protein component Ffh (fifty four homolog) and the regulatory 4.5S RNA [2-4]. Ffh contains two functional domains, i. e. the M and NG domain, which are connected via a flexible linker. The M domain recognizes signal sequences of nascent membrane proteins and binds the 4.5S RNA by a helix-turn-helix motif [5,6]. The NG domain harbors a GTPase activity and interacts with the membrane-anchored SRP receptor FtsY [7]. GTP-dependent interaction of SRP and FtsY allows release of nascent membrane proteins to the protein-translocation complex SecYEG [7-9].
We found that in *E. coli* the cytosolic AAA+ protease Lon is involved in regulation of protein translocation because Ffh is growth phase-dependently degraded by Lon. In order to determine the recognition mechanism of Ffh by Lon we seperatly analyzed the stability of the two functional Ffh domains *in vivo*. While the NG domain was stable, the M domain was degraded over the entire growth curve, respectively. Thus the M domain is recognized by Lon. These results are first evidence for the involvement of the AAA+ protease Lon in regulation of membrane protein transport and membrane integrity.

References
[1] Powers T, and Walter P (1997). EMBO J; 16(16); 4880-6.
[2] Ribes V, Römisch K, Giner A, Dobberstein B, Tollervey D (1990). Cell; 63(3); 591-600.
[3] Poritz MA, Bernstein HD, Strub K, Zopf D, Wilhelm H, Walter P (1990). Science; 250(4984); 1111-7.
[4] Luirink J, High S, Wood H, Giner A, Tollervey D, Dobberstein B (1992). Nature; 359(6397); 741-3.
[5] Zopf D, Bernstein HD, Johnson AE, Walter P (1990). EMBO J; 9; 4511-17.
[6] Keenan RJ, Freymann DM, Walter P, Stroud RM (1998). Cell; 94(2); 181-91.
[7] Miller JD, Bernstein HD, Walter P (1994). Nature; 367(6464); 657-9.
[8] Peluso P., Shan SO, Nock S, Herschlag D, Walter P (2001). Biochemistry; 40(50); 15224-33
[9] Angelini S, Deitermann S, Koch HG (2005). EMBO Rep; 6(5); 476-81

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

566/MTP
The role of the anaerobic C4-dicarboxylate-transporters DcuA, DcuB and DcuC on transport and regulation in *Escherichia coli*
A. Strecker*[1], G. Unden[1]
[1]*Johannes Gutenberg-University, Institute for Microbiology and Wine Research, Mainz, Germany*

Escherichia coli metabolizes C4-dicarboxylates during aerobic and anaerobic growth. Under anaerobic conditions the uptake of C4-dicarboxylates is catalyzed by the three transporters DcuA, DcuB and DcuC [1]. DcuB is a bifunctional protein. It forms a complex with the sensor kinase DcuS of the DcuSR two-component system and functions as a coregulator for DcuSR dependent gene expression under anaerobic conditions. Complex formation of DcuB with DcuS is required to convert DcuS to the C4-dicarboxylate responsive form [2]. Deletion of DcuB causes constitutive expression of DcuS regulated genes.
Complexome profiling of membrane proteins, BACTH and mSPINE interaction studies indicate that the transporters DcuA, DcuB and DcuC interact and form heterocomplexes. Formation and function of the Dcu heterocomplexes in transport and regulation will be discussed.

References
[1] Unden, G., Wörner, S., & Monzel, C. (2016). Adv. Microb. Physiol. 68, 139-167
[2] Wörner, S., Strecker, A., Monzel, C., Zeltner, M., Witan, J., Ebert-Jung, A., & Unden, G. (2016). Environ Microbiol. doi: 10.1111/1462-2920.13418

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

567/MTP
CyaC - a redox controlled Adenylate cyclase from *Sinorhizobium meliloti*
J. Wissig*[1], G. Unden[1]
[1]*Johannes-Gutenberg University Mainz, Institute for Microbiology and Wine Research , Mainz, Germany*

Adenylate cyclases (AC) are widespread in all kingdoms of life, including eukarya, bacteria and archaea. The production of the second messenger cAMP from ATP plays a role in regulating cell metabolism and differentiation. Based on sequence differences in the catalytic domain, the adenylate cyclases are grouped in 6 classes [1]. Class III ACs are common in eucaryotes and prokaryotes, but function and regulation of the prokaryotic class III AC has not been characterized in detail [2]. The bacterial adenylate cyclase CyaC belongs to the subgroup IIIb and is one of 27 further adenylate cyclases in *Sinorhizobium meliloti*.

CyaC$_{Sm}$ is membranebound by a 6TM-membrane anchor that resembles the anchor of eukaryotic class-III ACs. The CyaC membrane anchor contains, in addition, a conserved heme-binding site similar to that of bacterial di-heme-succinate-quinone-oxidoreductases (SQOR) [3]. The c-terminal catalytic domain of the cyclase is linked to the transmembrane domain by an 2Fe2S-cluster. The activity of CyaC is controlled by the cellular redox state.

References

[1] Linder JU (2006) Class III adenylyl cyclases: molecular mechanisms of catalysis and regulation. *Cell Mol Life Sci. 63(15):1736-51.*
[2] Schultz JE and Natarajan J (2013) Regulated unfolding: a basic principle of intraprotein signaling in modular proteins. *Trends Biochem Sci. 38(11):538-45.*
[3] Schirawski J and Unden G (1998) Menaquinone-dependent succinate dehydrogenase of bacteria catalyzes reversed electron transport driven by the proton potential. *Eur J Biochem. 257(1):210-5.*

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

568/MTP

Functional Characterization of the C4-dicarboxylate Transporter DcuB2As of *Actinobacillus succinogenes*

M. N. Rhie*[1], O. B. Kim[1,2]
[1]*Ewha Womans University, Interdisciplinary Program of EcoCreative, The Graduate School, Seoul, South Korea*
[2]*Ewha Womans University, Department of Life Science, Seoul, South Korea*

Introduction: *Actinobacillus succinogenes* is a rumen bacterium known as a natural succinate producer during C4-dicarboxylate fermentation. *A. succinogenes* possesses several potential C4-dicarboxylate transporters; among them, DcuB2As (the second DcuB of *A. succinogenes*) is more highly expressed than other transporters. DcuB2As is similar to that of DcuBEc (DcuB of *Escherichia coli*), but there is notable difference in amino acid sequence. The DcuB2As is about 100 amino acids longer than that of DcuBEc, which is intercalated in the middle of the amino acid sequence and indicated as IHD (Intercalating Hydrophilic Domain).
Objectives: This study aims to characterize the function of the C4-dicarboxylate transporter DcuB2As.
Materials & methods: Two fusion proteins were constructed: IHD was from DcuB2As deleted (DcuB2AsΔIHD), and IHD was into DcuBEc inserted (DcuBEc::IHDDcuB2As). The transport activities of proteins DcuB2As, DcuBEc, DcuB2AsΔIHD, and DcuBEc::IHDDcuB2As were detected in *E. coli* mutant containing no anaerobic C4-dicarboxylate transporters. The radiolabeled 14C-succinate and 14C-fumarate were used in this study.
Results and Conclusion: *E. coli* mutant containing DcuB2As grew faster and produced more succinate, less lactate and ethanol than that containing DcuBEc during anaerobic growth on fumarate plus glycerol. The fumarate uptake activity of DcuB2As was two-fold higher than DcuBEc in high substrate concentration (> 1mM), whereas succinate uptake of DcuB2As was similar to DcuBEc. The absence of presence of IHD in DcuB2As or DcuBEc showed no significant effect on uptake for fumarate. In addition, DcuB2As imported fumarate with exchange of succinate efflux. In conclusion, DcuB2As of *A. succinogenes* is a succinate/fumarate antiporter whose fumarate uptake activity is higher than DcuBEc of *E. coli*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

569/MTP

Exploring membrane microdomains in the plant pathogen *Agrobacterium tumefaciens*

S. Czolkoss*[1], X. Safronov[1], S. Rexroth[2], M. Aktas[1]
[1]*Ruhr-Universität Bochum, LS Biologie der Mikroorganismen, Bochum, Germany*
[2]*Ruhr-Universität Bochum, PG Cyanobakterielle Biotechnologie, Bochum, Germany*

The organization of bacterial membranes has received considerable attention over the past years. A number of studies on the hetero-spatial distribution of membrane lipids and proteins revealed structures of molecular disorder similar to eukaryotic lipid rafts (1). These microdomains are involved in various membrane-associated processes, such as signaling and membrane trafficking (1, 2). Proteins of the SPFH group (stomatin/prohibitin/flotillin/HflK/C) are bona-fide markers for the presence of raft-like structures. Our work on the membrane organization of the plant pathogenic bacterium *Agrobacterium tumefaciens* has revealed the presence of three SPFH proteins, termed HflK, HflC and Atu3772. Mass spectrometry-based analysis showed that these proteins are strongly enriched in detergent-resistant membrane (DRM) fractions generally considered to contain the lipid raft compartment of membranes. In addition, a number of proteins involved in membrane trafficking and quality control are associated with DRMs, such as outer membrane proteins, subunits of ABC transporter and the membrane-bound protease FtsH. Under virulence-induced conditions, proteins of the type IV and type VI secretion systems localize to DRMs suggesting the involvement of membrane microdomains in the infection process. Protein localization analysis indicated that the SPFH proteins HflK, HflC and Atu3772, as well as FtsH show a patchy distribution along the bacterial membrane.

References

[1] Lopez D, Kolter R (2010). Functional microdomains in bacterial membranes. Genes Dev. 24:1893-1902
[2] Mielich-Suss B, Schneider J, López D (2013). Overproduction of flotillin influences cell differentiation and shape in *Bacillus subtilis*. MBio 4:e0071900713

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

570/MTP

Structural studies of the complete mitochondrial ATP synthase by using a combination of X-ray crystallography and cryo-electron microscopy

K. Parey*[1], A. Hahn[1], M. Bublitz[2], D. Mills[1], V. Zickermann[3], J. Vonck[1], W. Kühlbrandt[1], T. Meier[4]
[1]*Max Planck Institute of Biophysics, Department of Structural Biology, Frankfurt/Main, Germany*
[2]*University of Oxford, Department of Biochemistry, Oxford, United Kingdom*
[3]*Goethe University Frankfurt, Institute of Biochemistry II, Frankfurt/Main, Germany*
[4]*Imperial College London, Department of Life Sciences, London, United Kingdom*

The inner mitochondrial membrane contains dimers of F_1F_o-ATP synthase, which by rotary catalysis produces most of the adenosine triphosphate (ATP) in the cell. The proton-motive force across the inner membrane drives the *c*-ring rotor in the membrane-embedded F_o subcomplex, generating torque that powers a sequence of conformational changes in the membrane-extrinsic F_1 subcomplex and resulting in ATP generation [1,2,3]. The F_1c_{10} subcomplex consists of the membrane-embedded c_{10} ring connected with the catalytic F_1 headgroup via a central stalk. We solved the crystal structure of the yeast *Yarrowia lipolytica* F_1c_{10} subcomplex by X-ray crystallography to a resolution of 3.5

Å [4]. Despite the absence of inhibitors used in the crystal setup, the structure shows the catalytic sites of the *β*-subunits in three different conformations, which correspond to a late post-hydrolysis state. The 3.5 Å *YL*F_1c_{10} crystal structure fits well into the density of the cryo-electron microscopy map, which was solved to a resolution of 6.2 Å and resolved 58 of 60 dimer subunits. The latter explains the molecular basis of ATP synthase dimerization and provides novel insights of cristae formation in mitochondria as a key feature of eukaryotic cell morphology [4].

References

[1] Abrahams, J.P., Leslie, A.G.W., Lutter, R., and Walker, J.E. (1994). Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. Nature *370*, 621-628
[2] Boyer, P.D. (1997). The ATP synthase--a splendid molecular machine. Annu Rev Biochem *66*, 717-749
[3] Noji, H., Yasuda, R., Yoshida, M., and Kinosita, K. (1997). Direct observation of the rotation of F1-ATPase. Nature *386*, 299-302
[4] Hahn, A., Parey, K., Bublitz, M., Mills, D.J., Zickermann, V., Vonck, D.J., Kühlbrandt, W., and Meier, T. (2016). Structure of a complete mitochondrial ATP synthase dimer reveals molecular basis of inner membrane morphology. Mol Cell, *63*(3), 445-456

Fig.1: The X-ray crystallographic structure of the yeast *Yarrowia lipolytica* F_1c_{10} ATP synthase subcomplex at 3.5 Å.
Fig.2: The structure of the *Y. lipolytica* ATP synthase dimer solved by single-particlecryo-electron microscopy.

Figure 1

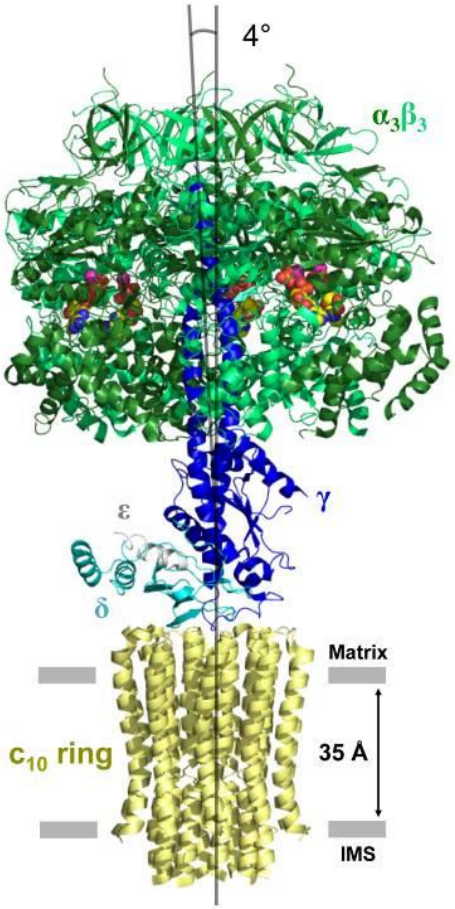
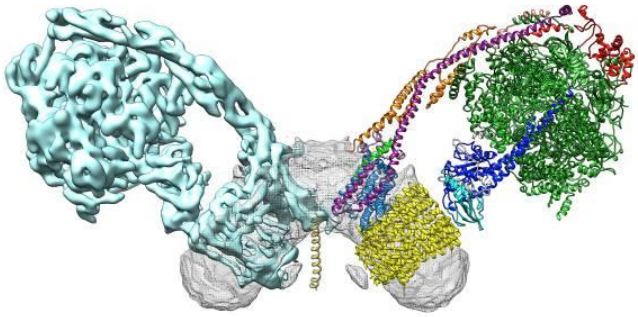


Figure 2



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

571/MTP

Mechanistic insights into foldase assisted folding of lipase from *Pseudomonas aeruginosa*

P. Dollinger[1], J. Kubiak[2], N. Verma[3], A. Viegas[4], F. Kovacic*[1], M. Etzkorn[4], H. Gohlke[3], C. Seidel[2], K. E. Jaeger[1]
[1]*Institute of Molecular Enzyme Technology, Heinrich Heine University Düsseldorf, Jülich, Germany*
[2]*Heinrich-Heine-University of Düsseldorf, Molecular Physical Chemistry, Düsseldorf, Germany*
[3]*Heinrich-Heine-University of Düsseldorf, Institute of Pharmaceutical and Medicinal Chemistry, Düsseldorf, Germany*
[4]*Heinrich-Heine-University of Duesseldorf, Institute of Physical Biology, Düsseldorf, Germany*

Proper folding and localisation of proteins are of extraordinary importance for their function within each living cell. We have studied the lipase LipA produced by the Gram-negative human pathogenic bacterium *Pseudomonas aeruginosa.* The function and cellular localization of this enzyme is controlled by a complex system including the cytoplasmic membrane-bound chaperone Lif, the SecYEG complex and the Xcp-machinery. While SecYEG and Xcp complexes, respectively, assist the transport of LipA across the inner and outer membrane, Lif has a function for conversion of LipA into an enzymatically active conformation[1]. Binding and activation of LipA by Lif is accompanied by an increase of alpha helical structures in the Lif indicating notable dynamics upon the formation of the Lif-LipA complex[2].
To study the mechanism of action of the highly flexible chaperone Lif upon LipA folding and secretion we have combined biochemical, fluorescence (single molecule FRET and fFCS), all-atom molecular dynamics (MD) and NMR methods. Biochemical analysis and single molecule FRET measurements of Lif:LipA complexes revealed a highly stable structure, which resembles the static structure predicted by homology modelling using the X-ray structure as a template. In the absence of LipA, Lif undergoes reversible conformational changes at different timescales, which can be interpreted as binding and release of LipA. Furthermore, FRET and MD results with unbound Lif revealed unfolding of an α-helix located in the C-terminal domain of Lif. This structural motif is important for LipA folding as shown by site directed mutagenesis. We solved by NMR spectroscopy the 3D structure of an N-terminal domain of Lif harbouring a strongly conserved foldase sequence motif essential for LipA activation. Although, the overall structure of the N-terminal domain is similar to the one predicted by the homology model of Lif, the observed differences provide the basis for understanding the binding of LipA by Lif. Conclusively, we have identified distinct structural motifs in Lif which presumably affect dynamics of multiple Lif conformations important for LipA activation process.

References

[1] Rosenau F and Jaeger K-E (2000) Biochemie 82: 1023-1032.

[2] Pauwels K *et al.* (2006) *Nature* 13: 374-375.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

572/MTP
From magnetosome membrane invagination to iron transport – the dual role of MamB in magnetosome formation
R. Uebe*[1], O. Raschdorf[1], J. M. Plitzko[2], D. Schüler[1]
[1]*Universität Bayreuth, Bayreuth, Germany*
[2]*MPI für Biochemie, Planegg-Martinsried, Germany*

Magnetotactic bacteria (MTB) are a unique group of prokaryotes that synthesize specialized organelles, so-called magnetosomes, for magnetic navigation. In the model MTB *Magnetospirillum gryphiswaldense* magnetosomes consist of membrane-enclosed magnetite (Fe_3O_4) nanoparticles that are aligned into a single coherent chain by dedicated cytoskeletal structures. The formation of these unique organelles is a complex process that comprises several key steps that are governed by magnetosome-associated proteins. One of the most important proteins during this process is the cation diffusion facilitator (CDF) family member MamB. Initially implicated in magnetosome-directed iron transport, it was also shown to be required for magnetosome membrane invagination from the cytoplasmic membrane but its precise role remained elusive. In this study, we employed a multi-disciplinary approach to analyze MamBs role during magnetosome formation in detail. Using site-directed mutagenesis complemented with structural analyses, fluorescence microscopy, and cryo electron tomography we show that MamB is an active magnetosome-directed transporter with two distinct, essential functions. First, MamB is required for magnetosome vesicle formation. Second, MamBs transport activity is essential for magnetite nucleation but not required for vesicle formation. Furthermore, we determined the crystallographic structure of MamB cytosolic terminal domain and showed that it shares similarities with the cation diffusion facilitator family. Additionally, we provide evidence that magnetosome chain formation is mainly driven by biotic factors and does not require magnetic interactions. Together our data indicate that MamB is a bifunctional protein that has two distinct roles at magnetosome formation

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

573/MTP
Effect of protein phosphorylation on the archaellum regulatory network of S*ulfolobus acidocaldarius*
M. F. Haurat[1], S. V. Albers[1], L. F. Bischof*[1,2]
[1]*Albert-Ludwigs Universität Freiburg, Biologie II, Mikrobiologie, Freiburg, Germany*
[2]*Spemann Graduate School of Biology and Medicine (SGBM) , University of Freiburg, Freiburg im Breisgau, Germany*

Introduction: In the archaeon *S. acidocaldarius* expression of the motility structure, the archaellum, is induced by starvation employing a network of positive and negative regulators[1,2]. Recently, two membrane bound one-component systems named ArnR and ArnR1 were identified to be indispensable for motility in *S. acidocaldarius* and that a highly complex network, which also involves reversible protein phosphorylation, is involved motility regulation[1,3].
Objective: We aim to decipher the mechanism underlying motility activation in *S. acidocaldarius* and elucidate the precise function of ArnR and ArnR1 in the regulatory cascade underlying archaellum expression.
Methods: The effect of deletion and truncation of ArnR and ArnR1 on motility in *S. acidocaldarius* was investigated via motility assays and Western-blot analysis. *In vitro* phosphorylation assays were performed to study phosphorylation of ArnR and ArnR1. EMSA was performed to characterize the DNA-binding properties of ArnR and ArnR1 and to identify their target. *S. acidocaldarius* lipids were isolated and processed to liposomes, in which ArnR and ArnR1 were reconstituted in order to analyze their function in their native environment.
Results: The deletion of ArnR and ArnR1 had a severe effect on motility of *S. acidocaldarius*. Both proteins were identified to bind to a specific region in the archaellum operon and to be phosphorylated *in vivo*. Reconstitution of both proteins into *S. acidocaldarius* derived liposomes was performed successfully1.
Conclusion: Taken together our results deepen the knowledge about the highly sophisticated phosphorylation-dependent network that regulates motility of *S. acidocaldarius*.

References
[1] Lassak, K., E. Peeters, S. Wrobel & S.V. Albers, Mol. Microbiol. 88(1), 2013, p: 125-139
[2] Reimann, J., Lassak K., Khadouma S., Ettema T.J., Yang N., Driessen A.J., Klingl A. & Albers, S.V., Mol. Microbiol. 86(1), 2012, p. 24-36
[3] Hoffmann, L., Schummer, A., Reimann, J., Haurat, M. F., Wilson, A. J., Beeby, M., Warscheid, B. *and* Albers, S.-V. *(*2016*),* Expanding the archaellum regulatory network – the eukaryotic protein kinases ArnC and ArnD influence motility of Sulfolobus acidocaldarius. MicrobiologyOpen, 00*:* 1–14. *doi:* 10.1002/mbo3.414

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

574/MTP
Characterization of the *Helicobacter pylori* Cag typeIV secretion system proteins CagN and CagM
S. Bats*[1], D. Pscheniza[1], C. Bergé[2], L. Terradot[2], C. Josenhans*[1,3]
[1]*Hannover Medical School, Institute for Medical Microbiology, Hannover, Germany*
[2]*Université de Lyon 1, Institut de Biologie et Chimie des Protéines, Lyon, France*
[3]*German Centre for Infection Research DZIF, Partner site Hannover-Braunschweig, Hannover, Germany*

Most virulent *Helicobacter pylori* strains contain the *cag* pathogenicity island (*cag*PAI) which codes for about 30 proteins forming a type IV secretion system that translocates the effector CagA into host cells. While CagA and some other proteins have been extensively studied, other *cag*PAI proteins remain poorly characterized or are of unknown function, such as the proteins HP0538 (CagN) and HP0537 (CagM). Both proteins do not have detectable homologs in other type IV secretion systems and have a yet unknown function in the type IV secretion system. CagN-deficient *H. pylori* strains are still able to translocate CagA and show a proinflammatory phenotype on cells while CagM-deficient strains are not. CagM has recently been suggested to be part of the type IV secretion system core complex[1], seems to be membrane-located and is poorly soluble.
We are interested in characterizing these two *cag*PAI proteins biochemically and functionally. Furthermore, we have addressed the question, whether CagN is able to interact with other *cag*PAI proteins including CagM, which is presumably expressed in the same operon together with CagN.
His-tagged CagN was overexpressed as a full length protein, a cysteine-deficient mutant and a C-terminally truncated variant in *E. coli*. CagN proteins were purified natively in a three-step procedure. Finally, we obtained high amounts of soluble ultra-pure tag-free protein. Hisx6-CagM WT was also expressed in *E. coli* and purified by Ni2+ affinity. Ultrapure CagN and CagM were characterized by various biochemical and biophysical methods. CagN is nearly exclusively monomeric, while CagM is detected only as dimer and higher order multimers. Small angle X-ray scattering data indicates that CagN has an elongated shape

while CagM appears to be more globular. The two proteins interact with each other, and CagM self-interacts. Purification, characterization, copurification and interaction studies will be discussed in this presentation. In conclusion, we have identified a novel interaction between two *cag*PAI proteins with different shapes, which will be the basis of future functional research.

References
[1] Frick-Cheng, Pyburn, Voss, McDonald, Ohi, Cover, Molecular and Structural Analysis of the *Helicobacter pylori cag* Type IV Secretion System Core Complex, MBio, 2016

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Proteomics and Metabolomics (FG PM)

575/PMP

Metabolic and regulatory adaption of *Staphylococcus aureus* to different carbon sources

A. Giese*[1], P. Westhoff[2], D. Zühlke[1], K. Dörries[2], K. Methling[2], C. Liang[3], B. Lazazzera[4], M. Lalk[2], S. Engelmann[5], S. Fuchs[6], M. Hecker[1], K. Riedel[1], T. Dandekar[3], J. Pané-Farré[1]
[1]*Ernst-Moritz-Arndt-Universität Greifswald, Institute of Microbiology, Greifswald, Germany*
[2]*Ernst-Moritz-Arndt-Universität Greifswald, Institute of Biochemistry, Greifswald, Germany*
[3]*Julius-Maximilians-Universität Würzburg, Department of Bioinformatics, Würzburg, Germany*
[4]*University of California, Department of Microbiology, Immunology and Molecular Genetics, Los Angeles, California, United States*
[5]*Helmholtz Institute for Infection Research, Department of Microbial Proteomics, Braunschweig, Germany*
[6]*Robert Koch-Institut, Division Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany*

Objectives: *Staphylococcus aureus* generally resides as a harmless commensal on the mucous membranes of the nares and on the skin of humans. However, in the immunocompromised host or following penetration of mucosal and skin barriers, *S. aureus* can switch to a more aggressive lifestyle often causing severe or even life-threatening infections. We hypothesize, that this switch is also modulated by the nutrients available to *S. aureus,* which in addition to providing energy and carbon may also act as a habitat identification signal, thereby controlling gene expression beyond the catabolic pathways required for the utilization of the respective carbon source.
For example, during invasive disease glucose may be a major carbon source in the blood stream, whereas pyruvate may correlate with a colonizing lifestyle since it is available in significant amounts in nasal secretion. Lactate for instance is produced in high amounts by proliferating T-cells at the site of infection and may therefore function as a signal of the hosts" state of immune defense. Finally, many intracellular pathogens utilize glycerol as a major carbon source during host cell invasion. Thus, intracellular survival of *S. aureus*, which is regarded as an important step in the development of chronic *S. aureus* infections, may also depend on the metabolism of glycerol.
Methods: For a better understanding of the *S. aureus* adaptation processes during life as a harmless commensal or as a pathogen during invasive disease, we analysed carbon source utilization of *S. aureus* strain COL using a combined metabolomic and proteomic approach. To this end, *S. aureus* was grown in a synthetic medium with glucose as major carbon source or combinations of glucose with either pyruvate, lactate or glycerol as alternative carbon source. To investigate the impact of carbon catabolite repression under these conditions, we included a catabolite control protein A (CcpA) mutant in our analysis.
Results and Conclusions: Quantitative data will be presented describing the protein repertoire and metabolic profile of *S. aureus* when grown with different carbon sources. Thus far, our data indicate that lactate consumption is not restricted by the presence of glucose, whereas pyruvate and glycerol are only used if the glucose level drops below a specific threshold. Furthermore, our data confirm the impact of CcpA on enzymes of the TCA cycle and, in addition, suggest a regulation of metabolic pathways of selected amino acids, like proline, glycine and glutamate, by CcpA. In contrast, CcpA has no effect on glycolysis and compared to *B. subtilis*, CcpA does not influence expression of enzymes of overflow metabolism. Moreover, preliminary data suggest that the available carbon sources modulate expression of the secretome and therefore may contribute to the direct interaction of *S. aureus* with its host.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

576/PMP

Comparative exoproteome analysis and redox imaging of *Staphylococcus aureus* isolates from different clonal complexes

M. Hillion*[1], B. Walther[2], L. Adrian[3], T. Semmler[4], J. Bernhardt[5], L. Vu van[1], H. Antelmann[1]
[1]*Freie Universität Berlin, Institute of Biology-Microbiology, Berlin, Germany*
[2]*Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*
[3]*Helmholtz Centre for Environmental Research, Department Isotope Biogeochemistry, Leipzig, Germany*
[4]*Robert Koch-Institut, Microbial Genomics, Berlin, Germany*
[5]*Ernst-Moritz-Arndt-Universität of Greifswald, Institute for Microbiology, Greifswald, Germany*

Introduction: *Staphylococcus aureus* is a commensal inhabitant of skin and nose but also an important opportunistic pathogen of humans and livestock frequently associated with antibiotic resistance. Studies on *S. aureus* sub-populations revealed that genomes are well conserved between isolates from the same lineages despite geographic, temporal and selective diversity. However, variation of hundreds of genes can occur between isolates from different lineages and these genes could be involved in interaction with host components.
Objectives*:* In this study, we aimed to investigate the diversity of secreted virulence factors and the differences in their ROS detoxification capacities in human and zoonotic *S. aureus* isolates from different clonal complexes to link virulence factor expression with the redox state of these strains.
Materials & Methods: We focused on the *S. aureus* clonal complexes (CC) 8 and CC22 as dominant human lineages, and CC398 as dominant livestock-associated MRSA (LA-MRSA) which is disseminating rapidly. To study the diversity of secreted virulence factors, we compared their extracellular proteomes using label-free LC-MS/MS analysis. A common protein database was created based on the DNA sequencing data and the PAN genome IDs. To get insights into the redox states and the ROS detoxification capacities, we used our novel bacilliredoxin-fused redox biosensor to analyse the changes of the bacillithiol redox potential across different isolates.
Results: A common pattern of secreted proteins could be observed between *S. aureus* isolates of the same clonal complex including known and novel virulence factors. However, striking differences in their secreted virulence factors were observed between isolates from different clonal complexes. The extracellular proteins were identified and quantified using gel-free LC-MS/MS analyses. The quantification of the changes in the BSH redox potential is subject of our current studies.
Conclusion: Successful epidemic *S. aureus* lineages have distinct exoproteomes and exhibit a high diversity in their secreted

virulence factors while a similar pattern was observed between isolates of the same clonal complex.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

577/PMP

An integrated functional genomics approach to unravel the *mode-of-action* of novel antiinfective compounds

C. Wolff*[1], J. Herrmann[2], J. Pané-Farré[1], D. Zühlke[1], R. Müller[2], K. Riedel[1]
[1]*Ernst-Moritz-Arndt-University, Institute of Microbiology, Greifswald, Germany*
[2]*Helmholtz Institute for Pharmaceutical Research Saarland, Helmholtz Centre for Infection Research, Department of Pharmaceutical Biotechnology, Saarbrücken, Germany*

Introduction: The widespread use of antibiotics has led to the accumulation of resistant bacterial pathogens, thus new antibacterial agents are urgently needed. The search for novel substances involves understanding of the molecular mechanism of action of the inhibitor and the bacterial response to the compound. Quantitative gel-free proteomics and complementary Omics-technologies, i.e. transkriptomics and metabolomics, have emerged as valuable tools to study the physiology of microbes under antibiotic stress conditions. The presented study aims at the comparative analysis of one natural product, i.e. chelocardin (CHD), and one structural modified compound of chelocardin, i.e. amidochelocardin (CDCHD).
Objectives: Until now, the antibiotic stress response of *P. aeruginosa* PAO1 to CHD and CDCHD has been characterized by MS-based proteome analysis. The corresponding protein signatures have been used to identify the cellular targets and the *mode-of-action* of CHD and CDCHD.
Materials & methods: *P. aeruginosa PA01* was exposed to increasing concentrations of CHD and CDCHD to determine their minimal inhibitory concentrations (MIC). Subsequently, for proteomic analyses, multiples of the MICs were tested in growth experiments to identify antibiotic concentrations that reduced the growth rate of the bacteria but did not inhibit growth completely in order to map the specific antibiotic stress response. The soluble protein fractions of *P. aeruginosa* PAO1, harvested 90 min after antibiotic treatment, were prepared and proteins identified using a gel-free LC-IMSE approach in combination with the Hi3 method for absolute protein quantification.
Results: Although each antibiotic showed an individual protein expression profile, signature proteins specific for a common drug target were identified. These subsets of proteins, whose expression levels are characteristic for a specific antibiotic treatment, have been designated as proteomic signature. The addition of CHD and CDCHD led to a slight increase in the amount of proteins involved in protein synthesis. In parallel, proteins responsible for motility and oxidative stress response accumulated at higher amounts compared to the untreated control. In contrast, proteins involved in electron transport, aerobic respiration and cofactor synthesis were negatively affected. Remarkably, in all experiments the *in vitro* antimicrobial activity of CDCHD against *P. aeruginosa* PAO1 was usually 2-4 times higher than that of the natural CHD.
Conclusion: The proteome analysis suggests that *P. aeruginosa* suffers from two different types of stress when exposed to CHD or CDCHD, protein biosynthesis inhibition and membrane damage, and responds by over-expressing stress-specific proteins, indicating a dual mechanism of action for chelocardins. In ongoing experiments the proteome analyses will be complemented by transcriptomic and metabolomic analyses.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

578/PMP

Photoautotrophic Cyanophycin Production: Metabolic engineering and process optimization using the native cyanophycin producer *Synechocystis* sp. PCC 6803

B. Watzer*[1], A. Trautmann[2], C. Posten[2], K. Forchhammer[1]
[1]*Interfakultäres Institut für Mikrobiologie und Infektionsmedizin Tübingen , Mikrobiologie/Organismische Interaktionen, Tuebingen, Germany*
[2]*Karlsruhe Institute of Technology, Institute of Process Engineering in Life Sciences, Karlsruhe, Germany*

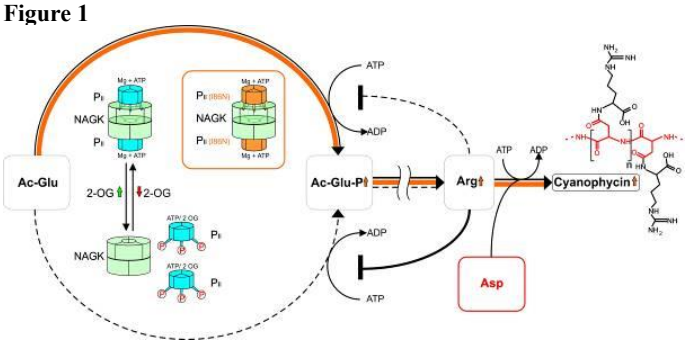
The PII signal transduction proteins are wide spread in prokaryotes and plants where they control the nitrogen assimilatory metabolism. A single point mutation in the PII signaling protein (I86N Variant) leads to a constitutive activation of the *N*-acetylglutamate kinase (NAGK), which is the key enzyme of the arginine biosynthesis (Fokina et al 2010). The unlock of the arginine pathway causes over accumulation of the biopolymer cyanophycin (multi-l-arginyl-poly-l-aspartate) (Watzer et al 2015). This biopolymer is of biotechnological interest as a source of amino acids and polyaspartic acid.
The PII (I86N) variant in *Synechocystis* sp. PCC6803 (strain BW86) strongly increases the *in vivo* activity of NAGK, leading to a more than 10 fold higher arginine content compared to the wild type. Due to the high intracellular arginine level, strain BW86 is able to accumulate up to 50-60% cyanophycin per cell dry mass, under tested conditions. As a consequence of the high cyanophycin amount, strain BW86 shows reduced PHB (polyhydroxybutyrate) accumulation under balanced growth conditions as well as under certain starvation conditions, indicating a relationship between the carbon storage polymer PHB and the carbon/ nitrogen storage polymercyanophycin in *Synechocystis*.
Cyanophycin accumulation is triggered by different stress conditions. For process optimization, phosphate limitation was figured out to accomplish the best biomass and cyanophycin production under batch cultivation in Midiplate photobioreactors. By evaluating cyanophycin and phosphor quotas, it was possible to determine the required phosphate amount for cyanophycin accumulation as well as the required phosphate amount to reach stationary growth phase. By cultivating the strain BW86 under defined conditions in photobioreactors, we were able to reach 340 g cyanophycin per m3 culture, resulting in 0.34 kg cyanophycin/kg cell dry mass within 9 days.

References

[1] Fokina O, Chellamuthu VR, Zeth K, Forchhammer K. 2010. A novel signal transduction protein P(II) variant from Synechococcus elongatus PCC 7942 indicates a two-step process for NAGK-P(II) complex formation. *J. Mol. Biol.* 399: 410-21
[2] Watzer B, Engelbrecht A, Hauf W, Stahl M, Maldener I, Forchhammer K. 2015. Metabolic pathway engineering using the central signal processor PII. *Microb Cell Fact* 14: 192

Figure 1 The strategy of metabolic engineering of the PII(I86N) protein in *Synechocystis* sp. PCC 6803 for arginine/ cyanophycin overproduction.

Figure 1



Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

579/PMP

Role of eukaryotic-type serine/threonine protein kinases (eSTPKs) in differentiation and spore wall synthesis of Streptomyces coelicolor A3(2)

B. Vollmer*[1], G. Muth[1]
[1]*University Tuebingen, IMIT, Tuebingen, Germany*

Streptomycetes are soil living bacteria of the Actinobacteria phylum. In contrast to most other bacteria which divide by binary fission, *Streptomyces* grows by apical tip extension forming a multiple branching mycelium. During morphological differentiation the aerial hyphae are transformed into spore chains by the simultaneous formation of dozens of sporulation septa. Septation and synthesis of the thickened spore wall involves the MreBCD proteins, which direct a rod-shaped morphology in other bacteria by positioning lateral wall synthesis.
Screening of a *S. coelicolor* genomic library by bacterial two-hybrid (B2H) analyses identified MreBCD interaction partners and led to the concept of the ***S****treptomyces* **s**pore wall **s**ynthesizing **c**omplex (SSSC). Interestingly, the SSSC also included PkaI, a putative eukaryotic-type serine/threonine protein kinase, which is encoded within a cluster of five independently transcribed eSTPK genes (*SCO4775-4779*).
The role of eSTPKs in bacteria and their influence on morphological differentiation and cell wall biosynthesis are not well characterized. However, the delay in sporulation and the presence of aberrant spores in NLΔpkaI and NLΔSCO4775–SCO4779 mutants suggests a regulation of the SSSC by protein phosphorylation. The specific phosphorylation of MreC and PBP2 by PkaI could be demonstrated in coexpression experiments.
In our project we want to elucidate the role of eSTPKs/phosphatases on the activity of SSSC proteins to understand whether protein phosphorylation is the key to control and coordinate differentiation in *Streptomyces*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

580/PMP

Proteomic analysis of organic sulfur compound utilisation in *Advenella mimigardefordensis* strain DPN7^T

C. Meinert*[1], U. Brandt[1], V. Heine[1], J. Beyert[1], S. Schmidl[1], J. H. Wübbeler[1], B. Voigt[2], K. Riedel[2], A. Steinbüchel[1]
[1]*Westfälische Wilhelms-Universität, Institut für Molekulare Mikrobiologie und Biotechnologie, Münster, Germany*
[2]*Ernst-Moritz-Arndt-Universität, Institut für Mikrobiologie, Greifswald, Germany*

Advenella mimigardefordensis strain DPN7^T was isolated because of its capability to utilise organic sulfur compounds such as the xenobiotic 3,3´-dithiodipropionic acid (DTDP), dibenzothiophene, taurine or 2-mercaptosuccinic acid (MS) as sole carbon source. Interestingly, *A. mimigardefordensis* strain DPN7^T is the only strain published that can metabolize DTDP as well as MS, which are of special interest since both compounds were discussed as precursor substrates for polythioester (PTE) production. The understanding of the metabolic network of this strain is mandatory for metabolic engineering, aiming for production of the polymer. Therefore, degradation of both organic sulfur compounds was investigated in this strain, applying proteome analyses, analyses of deletion mutants and enzyme assays. Protein extracts of cells cultivated with MS, DTDP or 3-sulfinopropionic acid (3SP) were compared with those cultivated with propionate (P) and/or succinate (S). Increased expressions of the chaperone DnaK (ratio DTDP/P 9.2, 3SP/P 4.0, MS/S 6.1, DTDP/S 6.2) and a Do-like serine protease (DegP) were detected during utilisation of all organic sulfur compounds. Furthermore, a putative bacterioferritin (locus tag MIM_c12960) showed strong expression (ratio DTDP/P 5.3, 3SP/P 3.2, MS/S 4.8, DTDP/S 3.9) and is probably involved in a thiol-specific stress response. The deletion of two genes encoding transcriptional regulators (LysR (MIM_c31370) and Xre (MIM_c31360)) in the neighborhood of the relevant genes of DTDP catabolism showed that these two regulators are essential for growth of *A. mimigardefordensis* strain DPN7^T on DTDP and that they most probably regulate transcription of genes mandatory for this catabolic pathway. Furthermore, proteome analysis revealed a high abundance (ratio MS/S 10.9) of a hypothetical cupin-2-domain containing protein (MIM_c37420). This protein shows an amino acid sequence similarity of 60 % to a newly identified MS dioxygenase from *Variovorax paradoxus* strain B4. Deletion of the gene and the adjacently located transcriptional regulator LysR, as well as heterologous expression of MIM_c37420, the putative mercaptosuccinate dioxygenase (Msdo) from *A. mimigardefordensis*, showed that this protein is the key enzyme of MS degradation in *A. mimigardefordensis* strain DPN7^T (K_M 0.2 mM, specific activity 17.1 µmol mg^{-1} min^{-1}) and under control of LysR (MIM_c37410).

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

581/PMP

Adaptation of *Dinoroseobacter shibae* to oxidative stress

N. Beier*[1,2], M. Kucklick[1,2], S. Fuchs[3], M. Behringer[4], E. Härtig[4], D. Jahn[4], S. Engelmann[1,2]
[1]*TU Braunschweig, Mikrobielle Proteomik, Braunschweig, Germany*
[2]*Helmholtzzentrum für Infektionsforschung, Braunschweig, Germany*
[3]*Robert Koch Institut, Wernigerode, Germany*
[4]*TU Braunschweig, Braunschweig, Germany*

The photoheterotrophic marine bacterium *Dinoroseobacter shibae* is a member of the highly abundant *Roseobacte*r group. Living in the photic zone of marine ecosystems *D. shibae* is frequently exposed to oxygen and an effective oxidative stress response is required. In the present study we analyzed the adaptation of *D. shibae* to different kinds of oxidative stress by using a GeLC-MS/MS approach. Exponentially growing *D. shibae* cells were exposed to 10 mM hydrogen peroxide, 15 µM paraquat or 500 µM diamide to induce peroxide, superoxide or disulfide stress. Altogether we identified and quantified more than 2300 cytosolic and surface-associated *D. shibae* proteins. The amount of 80 proteins changed significantly by at least 1.5 fold in response to peroxide stress, while 167 proteins where shown to be differently regulated by disulfide stress and 241 proteins by superoxide stress. Interestingly, the amount of only 5 proteins (GAPDH, KDPG-aldolase, Hsp33, RirA and Dshi_2021) was affected by all three stimuli while 395 proteins were specifically affected by one stimuli representing marker proteins for the respective stimulus. As expected different oxidative stress proteins like thioredoxin and peroxiredoxin were among the differently regulated proteins. Notably, the amount of primary scavenging proteins like catalase and superoxide dismutase did not change in response to oxidative stress. We assume that these proteins are constitutively highly expressed in *D. shibae* which might explain the observed high resistance of the bacterium to oxidative stress. Another interesting observation was that proteins involved in bacteriochlophyll biosynthesis pathways were repressed under disulfide and superoxide stress conditions. In contrast, proteins associated with iron transport accumulated in response to peroxide and superoxide stress. This is different to other bacteria doing the other way around to protect from high iron concentrations during oxidative stress. Interestingly, the amount of the rhizobial iron regulator RirA in *D. shibae* belonging to the proteins affected by all three stimuli was significantly reduced. A deletion mutant of *rirA* showed an improved adaptation to peroxide stress indicating that RirA dependent proteins may be important for oxidative stress

resistance. To elucidate the complete regulatory network responsible for the adaptation to oxidative stress we were also interested in the role of the three OxyR like proteins encoded by the genome of *D. shibae*. Preliminary results revealed that OxyR2 (Dshi_2523) might play a role during adaptation to oxidative stress, while OxyR1 (Dshi_0727) seems to be essential for growth under normal conditions.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

582/PMP

Water as universal marker of microbial activity: The incorporation of hydrogen and oxygen into the biomass of active organisms

S. Schäpe*[1], R. Starke[1], K. Olyphant[2], T. Sachsenberg[3], S. Baumann[1], O. Kohlbacher[3], E. Allen-Vercoe[2], N. Jehmlich[1], M. von Bergen[1]
[1]*Helmholtz Institut für Umweltforschung - UFZ, Molecular Systems Biology, Leipzig, Germany*
[2]*University of Guelph, Molecular and Cellular Biology, Guelph, Canada*
[3]*Universität Tübingen, Wilhelm Schickard Institut für Informatik, Tübingen, Germany*

Background: In the last decade, protein stable isotope probing (protein-SIP) has increasingly become more popular as a tool for quantitave assessment of metabolic activity, linking metabolic functionality and phylogenetic affiliation of active organisms in ecological systems (1). Commonly, a energy or nutrient source is labeled with the heavy stable isotope of carbon (^{13}C) or nitrogen (^{15}N) to unveil the atomic flow (2, 3). However, model compounds only scratch the surface of the actual ecological processes appearing in nature as multiple organisms are involved in the degradation of various compounds simultaneously. Labeling bacterial compounds with heavy water, either with D_2O or $H_2{}^{18}O$, could serve as a system-independent route to capture the entirety of microbial activity in all ongoing processes.
Method: The incorporation of D_2O and $H_2{}^{18}O$ was demonstrated for two concentrations and three time points each in the pure culture *E.coli* K12. Deuterium incoperation which leads to retention time shift of peptides during HPLC seperation was corrected *in silico*. Further, the incorporation of heavy water in a complex community was performed on the established "Robogut" system as a model fed with two different diets.
Results: Deuterium was abiotically incorporated into the acidic hydrogens in proteins due to the well-known HD-exchange. After sample preparation, only the deuteria in the backbone remain whereas the easily abstractable deuteria in the side chains are exchanged back to hydrogens. Otherwise, ^{18}O is biotically incorporated via different processes such as (A) the TCA cycle leading to all amino acids or (B) proteolytic cleavage of proteins.
Conclusion: In this study, the incorporation of heavy water is concentration- and time-dependent. As microbes necessarily require hydrogen and oxygen for biomass production, active microbes incorporate D and ^{18}O when supplied with heavy water. Hence, we present a universal marker for microbial activity regardless of the system and the energy or nutrient source.

References

[1] Jehmlich N, Vogt C, Lunsmann V, Richnow HH, von Bergen M. 2016. Protein-SIP in environmental studies. Current opinion in biotechnology 41**:**26-33.
[2] Starke R, Keller A, Jehmlich N, Vogt C, Richnow HH, Kleinsteuber S, von Bergen M, Seifert J. 2016. Pulsed (13)C2-Acetate Protein-SIP Unveils Epsilonproteobacteria as Dominant Acetate Utilizers in a Sulfate-Reducing Microbial Community Mineralizing Benzene. Microbial ecology 71**:**901-911.
[3] Starke R, Kermer R, Ullmann-Zeunert L, Baldwin IT, Seifert J, Bastida F, von Bergen M, Jehmlich N. 2016. Bacteria dominate the short-term assimilation of plant-derived N in soil. Soil Biol Biochem 96:30-38.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Infection Prevention and Antibiotic Resistance (FG PR)

583/PRP

Outbreak of Legionnaires' Disease in Warstein, Germany, 2013: Urinary antigen detection of *Legionella pneumophila* and *Streptococcus pneumonia*

C. Gagell*[1], L. Humpert[2], I. Menke-Möllers[3], C. Lück[1]
[1]*Institute of Medical Microbiology/TUD, Dresden, Germany*
[2]*Hospital "Maria Hilf" Warstein, Warstein, Germany*
[3]*MVZ synlab Leverkusen GmbH, Leverkusen, Germany*

Objective: In the summer of 2013, the city Warstein had to deal with the largest outbreak of Legionnaires" Disease (LD) in Germany till now. Seventy-eight laboratory-confirmed cases, including one fatality were determined. The epidemic strain, which could be isolated from seven LD patients as well as from environmental sources such as cooling towers, was identified as *Legionella pneumophila*, serogroup 1, subtype Knoxville, sequence type 345 [Maisa *et al.*, Euro Surveill. 2015]. The laboratory diagnosis of LD was based on two commercial urinary antigen tests. Furthermore, an in-house ELISA was performed for the direct subtyping of LD cases and *Legionella* negative cases were analyzed for *Streptococcus pneumoniae* infection.
Methods: A total of 508 urine samples from 315 persons suffering from pneumoniae during the LD outbreak (1 August to 6 September 2013) was analyzed using the Binax *Legionella* Urinary Antigen Enzyme Immunoassay (Binax EIA; Alere). Of these, 473 urines were also tested by the Sofia *Legionella* Fluorescence Immunoassay (FIA; Quidel). Urines with borderline values in the EIA were retested after a tenfold concentration using 5-kDa separators. Direct subtyping was performed with urines being strongly positive for *Legionella* antigen using monoclonal antibodies of the Dresden panel [Helbig *et al.*, 2012]. *Legionella* antigen-negative samples were analyzed by the immunochromatographic BinaxNow *Streptococcus pneumoniae* Antigen CARD (Alere).
Results: The Binax EIA revealed 99 *Legionella* antigen-positive samples corresponding to 62 patients. By means of the FIA 66 of 68 *Legionella* antigen-positive samples could be confirmed. However, after heating the samples 60 remained positive indicating unspecific reaction for the 6 samples. Interestingly, 10 of 338 *Legionella* antigen-negative urines in the Binax EIA were found to be positive based on the FIA results. Of the 19 samples with borderline values 15 were positive in the Binax EIA after urine concentration, while only 11 were confirmed by the FIA. To detect pneumococcal pneumonia 216 *Legionella* antigen-negative urines were tested by the *Streptococcus pneumoniae* BinaxNow assay revealing 8 *Streptococcus* antigen-positive urines after prolonged incubation of 60 min. The Knoxville subtype was identified in 17 of 18 LD cases using direct subtyping with MAb 3 and MAb 3/1 as detecting antibodies.
Conclusions: After urine concentration 7 more LD cases could be confirmed by Binax EIA and Sofia FIA, which were previously designated as *Legionella* antigen borderline. Since we also identified pneumococcal infections by the detection of *S. pneumoniae* antigen LD can be excluded at least for these patients. Furthermore, direct urinary antigen subtyping using monoclonal antibodies was successful.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

584/PRP

VirSaDB: A pangenome-derived database of virulence factors for an improved WGS-based characterization of *Staphylococcus aureus* isolates

S. Fuchs*[1], M. Nordwig[1], J. Bender[1], G. Werner[1,2], B. Strommenger[1,2]
[1]*Robert Koch Institute, Department of Infectious Diseases, Wernigerode, Germany*
[2]*National Reference Centre for Staphylococci and Enterococci, Wernigerode, Germany*

Introduction: Over the last decade, spectacular progress in whole genome sequencing (WGS) technologies has been made allowing the reconstruction of bacterial genomes on very high throughput. However, there is still a bottleneck in analyzing resulting data amounts with respect to identify, compare, and associate specific genetic determinants.
Objectives: The German National Reference Centre for Staphylococci and Enterococci (NRC) characterizes *S. aureus* isolates on a molecular level (i.) to provide insights into the structure of the *S. aureus* population in Germany and (ii.) to rapidly report about the emergence and prevalence of particularly resistant and virulent strain types. WGS-based surveillance and characterization of pathogens demand for high-quality databases comprising template sequences that are associated with virulence, resistance or clonal relatedness.
Methods: Extensive literature and database research was performed for the definition and selection of virulence gene targets. A pan-genome of 116 complete genome sequences of *S. aureus* (genomes provided by RefSeq) has been used to detect genetic variants which are now available in VirSaDB. To evaluate VirSaDB, 76 staphylococcal clinical isolates, sent to the NRC during February 2014, and 3 reference strains (8325, COL, N315) were sequenced utilizing Illumina MiSeq technology. Different *de novo* assemblers (A5-miseq, SPAdes, and Velvet) were used for genome reconstruction. Subsequently, Ridom SeqSphere+ Software was applied for VirSaDB target detection. Resulting virulence gene prediction was compared to results obtained from microarray analysis (Identibac *S. aureus* Genotyping Kit 2.0; Alere Technologies, Jena, Germany).
Results & Conclusion: In total, 329,332 annotated genes of 116 *S. aureus* strains have been grouped into 7,369 pan-genes using Proteinortho and PoFF (minimal identity 90%, minimal coverage 90%, equal weight of adjacencies vs. sequence similarity). Based on this, more than 7000 variants of 98 different *S. aureus* virulence genes have been integrated into VirSaDB. First results indicate a strong impact of the assembly algorithm on accurate gene detection using our evaluation dataset. The correlation of gene prediction with the output from microarray analysis will result in a workflow for feasible NGS-based virulence gene detection in *S. aureus*.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

585/PRP

Has been changed to 224/HYV.

586/PRP

Persistence and Dispersion of bacterial bioaerosols in a hospital washroom

J. Cooper*[1]
[1]*University of British Columbia, Occupational and Environmental Health, Vancouver, Canada*

Introduction: Nosocomial infections afflict one in twenty patients, killing millions of people every year and causing a significant financial burden to healthcare systems. One important but often overlooked source of nosocomial infections are washrooms in hospitals. Bioaerosols generated in hospital washrooms can remain airborne for minutes to hours, and potentially travel long distances with air currents. Healthcare workers and patients can be exposed to this potential hazard. One particularly important nosocomial pathogen is norovirus, which has shown significantly increased incidence in recent years. In this study, we used a bacterial and norovirus-surrogate inoculum to evaluate dispersal patterns of bioaerosol from a hospital toilet in an acute care facility.
Methods: *Escherichia coli* and *Enterococcus faecalis,* and the bacteriophage MS2 were used as the bacterial and norovirus-surrogate inoculums, respectively. Air samples were collected using the SAS duo 360 air sampler, at seven time points after toilet flushing and four locations in the bathroom. We also collected data on relative humidity and temperature, as well as took measurements of the local ventilation.
Results: All data has been collected and analysis is currently underway. Preliminary analysis shows significant bioaerosol concentrations at all locations until the 15-minute time point.
Conclusions: This work will provide information regarding the persistence and dispersion of bacterial and viral bioaerosols emanating from a hospital toilet. Specifically, data from the norovirus-surrogate will help inform healthcare facilities of the risk of exposure in washrooms for patients and healthcare workers to this prevalent nosocomial pathogen.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

587/PRP

Prevalence of multidrug-resistant organisms in refugee patients, medical tourists and domestic patients admitted to a German University Hospital

C. Reinheimer*[1], V. A. J. Kempf[1], K. Jozsa[1], T. A. Wichelhaus[1], M. Hogardt[1], F. O'Rourke[1], C. Brandt[1]
[1]*University Hospital Frankfurt, Institute for Medical Microbiology and Infection Control, Frankfurt, Germany*

Introduction: Patients with contact to healthcare-system in high-prevalence countries (HPC) and refugee patients in hospital settings (REF) have previously been identified to be at risk of carrying multidrug-resistant organisms (MDRO). Comparative studies addressing the epidemiology of MDRO in patients transferred from hospitals abroad (ABROAD) and REF are lacking but are necessary to introduce refined infection control measures. **Patients & Methods:** From December 2015 to June 2016, 117 REF, 84 ABROAD and 495 patients admitted to intensive care unit, with no refugee history or pre-treatment abroad (ICU), at University Hospital Frankfurt, Germany (UHF) were screened for MDRO on day of admittance. Data within these groups were compared and set in an epidemiological context.
Results: 52.1% (95% confidence interval=42.7-61.5) of REF and 41.6% (31.0-52.9) of ABROAD, were positive for at least one MDRGN, respectively. In contrast, 7.9% (5.6-10.6) of ICU were positive for MDRGN. Thereof, 0.9% (0.0-4.7) of REF, 15.5% (8.5-25.0) of ABROAD and 0% (0.0-0.7) of ICU were positive for at least one MDRGN with carbapenem resistance (CR). In total, 19 MDRGN with CR were detected in ABROAD, with the most frequent species with CR being *A. baumannii* with 42.1% (20.3-66.5). Regarding MRSA, 10.3% (5.4-17.2) of REF, 5.9% (1.9-13.3) of ABROAD and a significantly lower proportion 1.4% (0.6-2.9) of ICU, respectively, were tested positive.
Conclusions: Both REF and ABROAD pose a relevant hospital hygiene risk. High prevalence of MDRGN with CR in ABROAD was observed. Concise screening and infection control guidelines are needed in patient cohorts with increased risk for MDRO carriage.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

588/PRP

High prevalence of multidrug-resistant gram-negative organisms in HIV positive men

C. Reinheimer*[1], O. T. Keppler[2], C. Stephan[3], T. A. Wichelhaus[1], I. Friedrichs[4], V. A. J. Kempf[1]
[1]*University Hospital Frankfurt, Institute for Medical Microbiology and Infection Control, Frankfurt, Germany*
[2]*Max von Pettenkofer-Institute for Hygiene and Clinical Microbiology, Virology, München, Germany*
[3]*University Hospital Frankfurt, Department for Internal Medicine II/Infectious Diseases, Frankfurt, Germany*
[4]*University Hospital Frankfurt, Institute for Medical Virology, Frankfurt, Germany*

Background: Routes of transmission of multidrug-resistant gram-negative organisms (MDRGN) are not completely understood. Since sexual transmission of MDRGN might represent a potential mode which has not been noticed so far, this study evaluated rectal transmission of MDRGN in HIV positive men.
Methods: Between November 2014 and March 2016, we retrospectively investigated the MDRGN prevalence in rectal swabs of n=109 males tested positive for HIV (HP). These findings were compared to the MDRGN prevalence in n=109 rectal swabs in age-matched males tested negative for HIV (HN) within the same period. According to the hygiene plan of the University Hospital Frankfurt, Germany (UHF), patients admitted to intensive/intermediate care units are screened for MDRGN on day of admittance. Patients without HIV testing or MDRGN screening were excluded.
Results: MDRGN prevalence in rectal swabs was significantly higher in male HP (23.9%; 95 % confidence interval 16.2%–32.9%) than in age-matched male HN (8.3%; 3.8%–15.1%). In total, n=35 MDRGN species were detected, the most frequently was *Escherichia coli* with resistance due to ESBL expression and additional resistance to fluoroquinolones with n=25/35 (71.4%; 53.7.1%-85.3%). Of these, n=19/26 (73.1%; 52.2%–88.4%) were detected in HP and n=6/9 (66.7%; 29.9%–92.5%) in HN, respectively.
Conclusions: Reasons for higher rates of MDRGN in rectal swabs of male HP than in male HN are not understood but might indicate sexual transmission within the male HIV positive population. As therapy options for infections by MDRGN in particular are limited, prevention of MDRGN transmission should be emphasized.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

589/PRP

The BEAT-AMR consortium: Partnership against Biofilm-associated Expression, Acquisition and Transmission of Antimicrobial Resistance

F. Schreiber*[1], H. J. Kunte[1], Q. Ren[2], M. Buhmann[2], H. van der Mei[3], H. Busscher[3], S. Faust[4], J. Webb[5]
[1]*Federal Institute for Materials Research and Testing (BAM), Berlin, Germany*
[2]*Empa, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Biointerfaces, St. Gallen, Switzerland*
[3]*University Medical Center Groningen, Department of Biomedical Engineering, Groningen, Netherlands*
[4]*University of Southampton, Pediatric Immunology and Infectious Diseases and NIHR Wellcome Trust Clinical Research Facility, Southampton, United Kingdom*
[5]*University of Southampton, Biological Sciences, Southampton, United Kingdom*

Here, we introduce the BEAT-AMR consortium, which has been recommended for funding within the 3rd call of the Joint Programming Initiative on Antimicrobial Resistance (JPIAMR). The aim of the consortium is to investigate fundamental mechanisms that shape antimicrobial resistance in biofilms in relation to a biomaterial implant or device surface and translate those findings into clinical practice. We thereby aim to generate clinical recommendations on the combinatorial use of biomaterials coated with antimicrobials and antibiotics that avoid the occurrence and transmission of nosocomial biofilm infections with bacteria insusceptible to antibiotics. We established a Europe-wide network of experts in biofilm research, antimicrobial resistance, material sciences, and translational medicine that allows us to investigate those aspects in a coherent framework.
Biofilms are structured communities of bacteria found on surfaces that become embedded within a self-produced extracellular polymeric matrix. Biofilms can form on tissues or on biomedical surfaces, such as blood catheters or implants, where they act as a reservoir of potential healthcare-associated infection. Bacteria living in biofilms can tolerate much higher antibiotic concentrations compared to planktonic bacteria and survive long enough to evolve antimicrobial resistance (AMR). They form persistent, hard-to-treat infections and exhibit an intrinsic biology that promotes the development and transmission of AMR. The goal of our consortium is to determine how bacteria adapt to antimicrobials during biofilm formation on surfaces coated with antimicrobials, how AMR mutations are acquired and evolve within mature biofilms, and how population dynamics within biofilms affect the transmission of AMR. We address the hypothesis that understanding the contribution of biofilms to AMR acquisition and spread will lead to the development of novel antimicrobial strategies and medical devices that are more effective in preventing biofilm-associated infection and AMR. Our team provides facilities and clinical research governance for experimental and translational medicine. Our synergy of laboratory, clinical and translational research across Europe will ensure the best chance to develop novel and successful interventions and therapeutic outcomes.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

590/PRP

Has been changed into 809/MSP.

591/PRP

Xpert MTB/RIF for rapid detection of rifampicin resistance Mycobacterium tuberculosis from pulmonary tuberculosis patients in Southwest Ethiopia

M. T. Jano*[1], G. Abebe[1], D. Aragaw[1], L. Rigouts[2]
[1]*Jimma University, Mycobacteriology Research Center, Jimma, Ethiopia*
[2]*Institute of Tropical Medicine, Antwerp, Belgium*

Background: Accurate and rapid detection of drug resistant strain is critical for early initiation of treatment and for limiting the transmission of drug resistant TB. We investigated the accuracy of Xpert MTB/RIF for detection of rifampicin resistance and whether detection of rifampicin resistance by Xpert MTB/RIF predicts multidrug resistance (MDR) in Southwest Ethiopia.
Methods: Smear- or culture-positive sputa obtained from TB patients with increased suspicion of drug resistance were included in this study. GenoType MTBDRplus line probe assay (LPA) and Xpert MTB/RIF tests were done directly on smear-positive sputum specimens and on the cultured isolates for smear-negative specimens. We used the routine drug susceptibility test using LPA as the reference standard for confirmation of rifampicin (RIF) and isoniazid (INH) resistance.
Results: In this preliminary result, first line drug susceptibility results were available for 67 *M. tuberculosis* complex-positive sputum specimens using LPA test: 30% (20/67) were MDR-TB, 3% (2/67) were RIF monoresistant, 6% (4/67) were INH monoresistant, and 61% (41/67) were susceptible to both RIF and INH. Relative to routine RIF susceptibility testing (LPA), Xpert MTB/RIF detected all RIF resistance correctly with 100% sensitivity and 97.8% specificity. The positive predictive value of Xpert MTB/RIF for RIF resistance was 95.7%. Of 23 RIF resistant strains on Xpert MTB/RIF, 87% (20/23) were resistant to both RIF and INH (MDR), 8.7% (2/23) were RIF monoresistant, and 4.3% (1/23) were sensitive to RIF by LPA test. High proportion of RIF resistance was documented among patients previously categorized as failure cases (50%, 10/20) followed by relapse cases (31.6%, 6/19), and defaulters (28.6%, 2/7).
Conclusions: Xpert MTB/RIF was highly effective for identification of rifampin-resistant strains in smear or culture-positive samples. RIF resistance based on Xpert MTB/RIF result could be used to estimate multidrug resistance and can allow rapid initiation of MDR-TB treatment in regions with high drug resistant TB.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

592/PRP

New insights into the dark toxicity of the photosensitizer TMPyP in *E. coli*

D. Eckl*[1], L. Dengler*[1], M. Nemmert[1], A. Eichner[2], W. Bäumler[2], H. Huber[1]
[1]*University of Regensburg, Microbiology and Archaea Center, Regensburg, Germany*
[2]*University Hospital, Department of Dermatology, Regensburg, Germany*

Introduction: Nowadays, increasing antibiotic resistances of microorganisms pose a threat concerning the treatment of infections. Especially nosocomial infections acquired in hospitals or similar institutions exhibit such resistances causing serious problems.
Objectives: Photodynamic inactivation of bacteria (PIB) is a promising approach to treat infections like Methicillin resistant *Staphylococcus aureus* (MRSA). When a photosensitizer, the active compound in PIB, absorbs visible light, reactive oxygen species (ROS) arise and efficiently inactivate bacteria by a multi causal damage. The efficacy of PIB is usually evaluated by comparing bacterial survival with and without light irradiation. Any reduction of bacterial viability in the presence of photosensitizers without light is considered as dark toxicity, which is still controversially discussed for some photosensitizers like 5, 10, 15, 20-Tetrakis(1-methyl-4-pyridinio)porphyrin tetra(p-toluensulfonate) (TMPyP). TMPyP shows a high absorption of predominantly blue light and a high yield of ROS production. Thus, it might be difficult to evaluate dark toxicity of TMPyP under ambient light conditions under laboratory conditions.
Material and Methods: In the present study, different light spectra at low radiant exposures were applied to *Escherichia coli* to measure the effect of different ambient light conditions in the presence of increasing concentrations of TMPyP (from 0 to 250 µM). Serial dilutions of the treated cells were plated and grown colonies were plotted against the concentration of TMPyP for each light source.
Results: The results show that inactivation of bacteria only occurs at short wavelengths (around 420 nm), while no effect was detectable with green, yellow, or red light. Furthermore, experiments with low light intensities of natural sunlight indicate that dark toxicity is derived from residual light in the laboratory yielding a so-called dark control.
Conclusion: In summary we conclude that under adequate laboratory conditions, TMPyP exhibits **no** dark toxicity. Therefore, we recommend to define the conditions of dark controls no matter which organism or photosensitizer are used.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

593/PRP

Resistance profiles of community-acquired urinary tract infections in Germany (SARHA study)

A. Klingeberg[1], N. Willrich[1], M. Feig[1], I. Noll[1], K. Oberdorfer[2], A. Krenz-Weinreich[3], D. Emrich[4], W. Kalka-Moll[5], T. Eckmanns*[1], E. Zill[4]
[1]*Robert Koch-Institute, Berlin, Germany*
[2]*MVZ Labor Dr. Limbach, Heidelberg, Germany*
[3]*LADR GmbH Medizinisches Versorgungszentrum Plön, Plön, Germany*
[4]*Labor 28, Berlin, Germany*
[5]*Medizinisches Versorgungszentrum Dr. Stein & Kollegen, Mönchengladbach, Germany*

Introduction: Guidelines and treatment recommendations for calculated antimicrobial therapy of community-acquired uncomplicated urinary tract infections (UTI) are based on surveillance systems for routine data. However, these include mainly complicated UTI since uncomplicated UTI are rarely tested in clinical practice. Thus, the recorded resistance profiles might not be representative
Objectives: Our aim was to investigate the resistance profile of *Escherichia coli* against Trimethoprim (TRP) and Trimethoprim-Sulfamethoxazol (SXT) in community-acquired uncomplicated UTI and to compare it with data from the German Antibiotic-Resistance-Surveillance (ARS) system.
Materials and methods: Between July 2015 and February 2016 more than 1300 patients with UTI were enrolled by 59 general and internal practices in 6 different federal states of Germany. Urine analyses were performed to investigate the resistance profile and physicians completed a short questionnaire including the patients sex, age, and factors to differentiate UTI in complicated and uncomplicated. Resistance profiles of *E. coli* against TRP and SXT were compared to ARS data from the same practices 1 year earlier.
Results: By October 2016, 1291 samples were available for analysis. At all 811 (62.8%) patients had a positive urine culture, of which 599 (73.9%) were infected with *E. coli*. Resistance of *E.coli* against TRP was detected in 21.7% (95% CI: 18.2- 25.1) of the patients and against SXT in 17.9% (95% CI: 14.8- 21.0). In the subgroup of uncomplicated UTI, resistance of *E.coli* against TRP was found in 41 of 287 patients (14.3%, 95% CI 10.2 – 18.0), against SXT in 38 of 306 patients (12.4% 95% CI 8.7-16.1). Resistance to TRP in complicated UTI was found in 68 of 257 patients (26.5%, 95% CI 21.1- 31.9), and against SXT in 66

of 274 patients (24.1%, 95% CI 19.0- 29.2). In comparison, *E. coli* resistance measured in the ARS surveillance system between July 2014 and February 2015 was 24.3% against TRP and 23.7% against SXT in the same practices.
Conclusion: There is a substantial difference in resistance of *E. coli* between complicated and uncomplicated UTI. Surveillance systems are not representative for the resistance pattern in uncomplicated UTI. This has to be considered in recommendations for calculated antimicrobial therapy.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

594/PRP
Genetic Epidemiology of the *Enterobacter cloacae* Complex at a University Hospital over a 3-Years Period
S. Bletz*[1], B. Middendorf[1], A. Kossow[1], S. Kampmeier[1], D. Rubin[1], F. Schaumburg[2], A. Mellmann[1]
[1]*Institute of Hygiene, Muenster, Germany*
[2]*Institute of Medical Microbiology, Muenster, Germany*

Background: In recent years, members of the *Enterobacter cloacae* complex (ECC) have been identified as emerging pathogens, frequently associated with nosocomial infections. Many of these pathogens are resistant to traditional antibiotics and can develop resistances against new antimicrobial agents. Recently, several ECC outbreaks have been reported, especially in neonatal units. Different typing methods are established, e.g. pulsed-field gel electrophoresis, multilocus sequence typing (MLST) or *hsp60* gene sequencing to analyze the strain relatedness between outbreak isolates.
Aim: To elucidate the spread and subtyping of the ECC for infection control purposes, we used a genome-wide typing scheme based on gene-by-gene comparisons (core genome [cg]MLST) and compared this method with MLST and *hsp60* gene sequencing.
Method: We included ECC isolates of colonized or infected patients detected at the University Hospital Münster between October 2013 and September 2016. The isolates were categorized according to the multi-drug resistant Gram negative bacteria (MRGN) scheme as suggested by the KRINKO of the Robert Koch-Institute. The strains were whole genome sequenced (WGS) on an Illumina MiSeq sequencer. We performed sequence-based typing on three different levels (*hsp60* gene cluster, MLST, and cgMLST) using the Ridom SeqSphere+ software (Ridom GmbH; Münster, Germany). Subsequently, we compared the discriminatory indices (*DI*) of the typing methods and interpreted the results considering epidemiological data. Clustering of isolates was visualized by generating minimum spanning trees (MST) using the same software.
Results: During the investigation period of three years, we detected 83 isolates from 76 patients. The majority of these (n=72) exhibited a multi-drug resistant phenotype: 50 isolates were 3-MRGN, followed by 4-MRGN (*n*=20), and 2-MRGN (2). The detected isolates (only one isolate per patient, n=76) belonged to six different *hsp60* clusters: cluster I (*n*=2), cluster II (3), cluster III (20), cluster VI (1), cluster VIII (48) and cluster XI (2). The *DI* of the different typing methods ranged from 0.536 (95% CI 0.435 - 0.636; *hsp60*) with six different genotypes, over 0.958 (95% CI 0.939 - 0.976; MLST) with 35 STs, to 0.997 (95% CI 0.993 - 1.0; cgMLST) with 70 genotypes. Fourteen strains were isolated from patients on a single ward within a two-month period exhibiting ten different cgMLST genotypes. Eight isolates belonged to two clusters with four isolates each, which exhibited nearly identical cgMLST genotypes. The seven follow-up isolates of the same patients had identical cgMLST genotypes.
Conclusion: To characterize ECC, WGS and subsequent cgMLST is able to highly discriminatory and reproducibly characterize the diversity within this species thereby contributing to the implementation of efficient infection control strategies.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

595/PRP
Screening adherence and infection control practices in patients with Carbapenem-resistant *Acinetobacter baumannii* in a university hospital
C. Baier*[1], E. Ebadi[1], C. Lassahn[1], F. C. Bange[1]
[1]*Institut für Medizinische Mikrobiologie und Krankenhaushygiene, MHH, Hannover, Germany*

Question: Carbapenem-resistant (Cr) *Acinetobacter baumannii* (*Ab*) strains require sophisticated infection control practices, and treatment is challenging in case of infection.
Methods: A protocol for screening on admission for Cr gram-negative bacteria has been established in our clinic since 1/2015. This includes patients (pat) who are hospitalized domestically or abroad in the past year (risk factor based screening). We identified all patients with a first-time positive culture for Cr *Ab* (1/2015-9/2016). The adherence to the admission screening procedure (rectal and skin swabs) in this cohort was evaluated. Moreover we herein present our infection control practices regarding Cr *Ab*.
Results: Overall 16 pat were identified. 4 pat were previously tested positive in another clinic. 2 other pat acquired CR *Ab* by colonized donor organs. These patients were excluded from analysis. 6 of the remaining 10 pat had been hospitalized abroad, 3 pat were admitted from domestic hospitals and 1 pat had been hospitalized abroad and domestically. Thus *all* 10 pat had risk factors which should have triggered screening on admission. Adherence to admission screening was 2 of 10. The 2 pat who have been screened on admission were tested negative. They were tested positive later during the hospital stay, after carbapenem therapy had been started. From the 8 pat that were not screened correctly on admission, 3 pat were tested positive on day 1 or 2 of hospital stay, whereas 5 were tested positive on day 3 or later. Of these 8 pat, 5 received antibiotic therapy prior to positive results.
The following infection control practices are recommended in our clinic:

- Preemptive isolation and contact precautions for patients with hospitalization abroad until negative admission screening swabs are present.
- Quarantine for CR *Ab* contact patients.
- Prevalence screening once weekly for *all* patients on the ward as long as the index patient is present (this screening is repeated 1 and 4 weeks after discharge of the index patient).
- Octenidine-based antiseptic body washings for index patients.

Conclusions: The results of this small cohort analysis indicate low adherence to admission screening procedure. Negative results in the 2 patients that were screened on admission reflect the low sensitivity for screening of Cr *Ab* in rectal swabs. Most patients were eventually detected by specimens taken due to suspected infection. Even so adherence to screening on admission was low in our cohort none of the 16 patients produced a secondary case due to transmission, probably because of a high detection rate during the hospital stay. In summary, it may be necessary to screen pat with a negative or missed Cr *Ab* screening on admission later during the hospital stay, especially after antibiotic therapy has been started.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

596/PRP

Investigation of the mechanisms involved in tigecycline resistance in *Enterococcus* spp.

S. Fiedler*[1], J. Bender[1], M. Ruschig[2], C. Fleige[1], U. Geringer[1], I. Klare[1], A. Mischnik[3], N. Mutters[4], G. Werner[1]
[1]*Robert Koch-Institute, Division 13 Nosocomial Pathogens and Antibiotic Resistances, Wernigerode, Germany*
[2]*University of Brunswick – Institute of Technology, Brunswick, Germany*
[3]*University Medical Center Freiburg, Department Innere Medizin, Freiburg, Germany*
[4]*University Hospital Heidelberg, Department of Infectious Diseases, Heidelberg, Germany*

Introduction: Tigecycline (TGC) represents one of the last-line therapeutics to combat multi-drug resistant bacterial pathogens including VRE and MRSA. The National Reference Centre for Staphylococci and Enterococci at the RKI received 123 TGC-resistant *E. faecium* and *E. faecalis* isolates in recent years. Development of resistance to TGC in enterococci is dependent on multiple mechanisms of which ribosomal protection due to Tet(M) and the alteration of the TGC binding site due to mutations within *rpsJ* were described.
Objectives: The aim of this study was to investigate further alternative TGC resistance mechanisms, as the above mentioned are dispensable for TGC insusceptibility. Further, we aimed to evaluate the influence of tetracycline selective pressure on development of TGC-resistance.
Materials & methods: Next generation sequencing by means of Illumina technology was applied to reveal potential genomic differences between high and low-level TGC-resistant strains (n=3). Two isolates were further subjected to RNA-Seq and candidate gene expression was validated thereafter by RT-qPCR. TGC-sensitive strains, treated with TET and TGC, over a period of 33 days, were periodically tested for alterations in minimal inhibitory concentrations (MICs) to both antibiotics, respectively. Sequence analysis of known resistance genes *tet*(M) and *rpsJ* was subsequently done for the successor isolates by Sanger sequencing.
Results: Comparative genome analyses of three isogenic strains, that showed different levels of TGC MICs, revealed Tet(L) and Tet(M) as drug resistance proteins. Whole transcriptome sequencing (WTS) confirmed an up-regulation of the respective genes within a TGC-resistant strain following TGC-challenge compared to the isogenic non-treated control. Detailed comparative transcriptomic analysis additionally revealed alternative pathways which might impact the development of TGC resistance. Also, DNA sequencing of *tet*(M) and *rpsJ* yielded several nucleotide positions which possibly enhance TGC MICs associated with distinct amino acid substitutions. Interestingly, treatment of various isolates with either TET or TGC resulted in elevated TGC MICs and vice versa.
Conclusion: Our results confirmed previous studies that *tet*(M)/*tet*(L), as well as *rpsJ,* are involved in TGC resistance. However, as not all TGC-resistant isolates were tested positive for either of the known resistance mechanisms, WTS revealed significantly altered gene expressions which suggest further possibilities of enterococci to adapt to antibiotic selective pressure. Most importantly, we observed induction of TGC-resistance upon treatment of TGC-susceptible isolates with tetracycline. This finding is particularly worrisome with respect to the One Health perspective as antibiotic treatment of livestock could result in subsequent dissemination of multi-drug resistant zoonotic pathogens.

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

597/PRP

Development of an Electronic Early Warning Surveillance based on Syndromic Surveillance System (SSS) in I.R.Iran (2011-16)

M. M. Gouya[1], P. Hemmati*[1], J. Ahmadi[2], M. Nabavi[1], S. Samei[3], K. Seif Farahi[4], R. Baiat[2], M. Soroush[1]
[1]*Ministry of Health & Medical Education, Iranian Center for Communicable Diseases Control, Tehran, Islamic Republic Of Iran*
[2]*Faranam Company, IT, Tehran, Islamic Republic Of Iran*
[3]*Ministry of Health & Medical Education, Iranian Reference Health Lab (RHL), Tehran, Islamic Republic Of Iran*
[4]*Physician, Toronto, Canada*

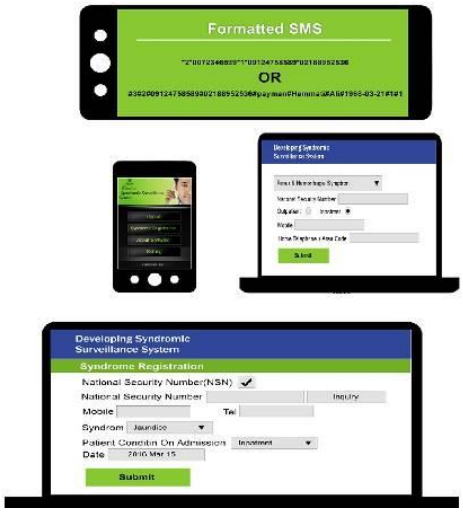
Question: Considering the emergence of Public Health Events of International Concern (PHEIC) such as Ebola, Pandemic Influenza, global health security is a growing concept. Some global authorities believe that WHO and its member states are not ready for next epidemics.
In order to be able to capture every PHEIC including emerging and reemerging diseases, Iran felt a high necessity to develop a surveillance system with the following main features:
EWAR (early detection) capacity Being sensitive even to those diseases emerging in the future Is not dependent on lab processing at the first step which might be time-consuming However, after receiving alerts out of SSS, it should be able to intelligently be integrated with conventional epidemiologic and lab-based surveillances to give a full picture of outbreak/epidemic.
Materials & Methods: What is meant by Iranian SSS is a case-based, indicator-based method in which health centers and relevant inter-sectoral organizations report clinical syndromes (data sources) (Image 1)
A list of clinical syndromes were organized and defined by Iranian CDC and a WHO mission to Iran in August 2011 (10 syndromes), which was expanded to 13 in a WHO workshop in Dubai in Dec.2011 and finally was finally increased to 14 syndromes and approved by Iranian CDC, Prof. David Heymann from PHE, UK and WHO country office colleagues in Turkey in January 2012.
Results: Iranian CDC and IT experts developed 4 main interlinked electronic components (sub-system) as such:

- Minimum data subsystem for EWAR (formatted sms, Android-based application and a web-based application) (Image 2)
- Maximum data subsystem for epidemiologic case investigation
- Laboratory subsystem
- Early detection smart engine

Discussions and Conclusion: International Health Regulations (IHR2005) requires all WHO member stated to detect any unusual/or unexpected health event in 24 hours and to do primary investigation/risk assessment and notify WHO in the next 48 hours.
Every syndrome is able to even capture emerging and remerging diseases because although the epidemiologic profile and threats might change through time (Pandemic flu, MERS-CoV, Ebola, Zika, next epidemics), yet the way they present is constant and is within the framework of a limited list of clinical syndromes (if symptomatic). Such advantage makes SSS even sensitive to diseases unknown to health workers and to laboratory setup in each country and helps WHO member states to prepare themselves and implement their requirements according to IHR2005 more effectively and adaptively.
I.R.Iran is the first country in Eastern Mediterranean Region (EMR) which has developed SSS and recently shared its experiences during a consultative workshop in Pakistan in Nov.2016 with seven EMR countries to develop an EWAR surveillance for Aedes-borne diseases.

Figure 1

Figure 2



Presentation on Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

598/PRP

Methicillin Resistant *Staphylococcus aureus* among HIV positive Pediatric Patients in Northwest Ethiopia: By GenoType MRSA Molecular Line Probe Assay

Y. Zenebe*[1,2], M. Tibebu[1,2], B. Tulu[1,2], D. Mekonnen[1,2], Z. Mekonnen[2,3]
[1]*Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Microbiology, Immunology and Parasitology, Bahir Dar, Ethiopia*
[2]*Bahir Dar University, Biotechnology Research Institute, Bahir Dar, Ethiopia*
[3]*Bahir Dar University, College of Medicine and Health Sciences, Department of Biochemistry, Bahir Dar, Ethiopia*

Background: Increasing evidence suggests that Methicillin resistant *Staphylococcus aureus* (MRSA) infections are becoming more prevalent throughout the HIV infected community. **Objectives:** This study was aimed to determine the prevalence of colonization by MRSA species among HIV positive pediatric patients in the Amhara National Regional State, Northwest Ethiopia.

Methods: Participants who attended the clinic from December 2013 through April 2014 were invited to participate in the study. Eligible participants were HIV-infected<18 years of age, receiving medical care at the Paediatric HIV clinics of Felege Hiwot, Dessie, and Debretabor Referral Hospitals. From each participant specimens for *S. aurous* culture were collected from the anterior nares, the skin of the back of the wrist and the perineum using sterile broth moistened swabs. Swabs were cultured and read according to standard microbiologic procedures. The GenoType MRSA VER 3.0 was used for characterization of *S. aurous* and *S. epidermidis* strains among culture positive patients by detecting methicillin resistance-mediating mecA & mecC genes. The bicomponent cytotoxin virulence factor Panton-Valentine leukocidin (PVL) were detected. Data was analyzed by descriptive and logistic regression model using SPSS version 20. The P value of <0.05 was considered as statistically significant.
Results: Among 202 culture positive patients, 126 (62.4%) were also confirmed by GenoType MRSA as S. *aurous* and of these, 47(37.3%) and 15(11.9%) were mecA and Panton-Valentine leukocidin genes positive respectively. There was an association between age and *S. aurous* colonization, (AOR=5.07, 95%CI, 1.10-23.41, P-value=0.03). The study sites, (AOR=4.90, 95%CI, 1.31-18.56, P value=0.01) and PVL gene, (AOR=7.51, 95%CI, 8.82-63.92, P value=0.001) have also significant association with MRSA colonization.
Conclusion and recommendation: High prevalence of pathogenic MRSA strains among HIV positive pediatric patients in the study area. From the PVL gene detection, most of MRSA type was HA MRSA. Hence, strict hygienic approaches by all healthcare workers in hospitals should be implemented to reduce the chance of hospital acquired MRSA infections.
Key words: MRSA, Pediatrics, HIV, Ethiopia

Presentation: Monday, 6 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Infection Immunology (FG II)

599/IIP

Differential activation of formyl-peptide receptors by *Staphylococcus aureus* and consequences for inflammation

E. Weiß*[1], D. Hanzelmann[1], F. von Loewenich[2], J. Liese[3], A. Klos[4], A. Peschel[1], D. Kretschmer[1]
[1]*Interfaculty Institute for Microbiology and Infection Medicine, University of Tübingen, Tübingen, Germany*
[2]*Institute for Medical Microbiology and Hygiene, Universitätsmedizin Mainz, Mainz, Germany*
[3]*Institute for Medical Microbiology and Hygiene, Uniklinikum Tübingen, Tübingen, Germany*
[4]*Department of Medical Microbiology and Hospital Epidemiology, Hannover Medical School, Hannover, Germany*

Formyl peptide receptors (FPR1-3) are crucial pattern recognition receptors governing leukocyte chemotaxis and cytokine release in response to microbe-associated molecular patterns (MAMPs). FPR1 senses formylated peptides produced by all kinds of bacteria, while FPR2 and 3 respond to certain endogenous peptides. In addition, we have recently demonstrated that phenol-soluble modulin (PSM) peptides from highly pathogenic *Staphylococcus aureus* are not only important cytolytic toxins but also highly efficient ligands for the human FPR2. Mouse neutrophils also respond to PSMs, but it has remained unclear, which of the mouse FPR paralogs senses staphylococcal PSMs.
To analyze the role of mouse FPRs, stably transfected RBL cells were generated, which either express mFpr1 or mFpr2. After stimulation with PSMs or culture filtrates of PSMs-secreting *S. aureus* strain USA300, we noticed strong calcium influx and degranulation in mFpr2-transfected cells, but no response in mFpr1-transfected cells and control cells. Moreover, by using

HoxB8 neutrophils, a primary neutrophil cell line prepared from wild-type (WT) and mFpr2 knockout mice (Fpr2-/-), we observed strong calcium influx, chemotaxis, MIP2 release and CD11b upregulation in wild-type HoxB8 but not in Fpr2-/- HoxB8 after stimulation with PSMs or culture filtrates of USA300.
These data indicate that the mouse Fpr2 is specifically activated by PSMs. Therefore, PSMs represent the first secreted MAMPs for the mouse Fpr2. Our data support the hypothesis that the mouse Fpr2 is the functional orthologue of the human FPR2 and that a mouse infection model may be a suitable model for analyzing the role of PSMs and FPRs during infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

600/IIP
Activation of the Contact System at the Surface of *Streptococcus gallolyticus* Represents a Possible Virulence Mechanism in Endocarditis
J. Isenring*[1], S. Dramsi[2], B. Kreikemeyer[1], S. Oehmcke-Hecht[1]
[1]*University Medicine Rostock, Rostock, Germany*
[2]*Institut Pasteur, Department of Microbiology, Paris, France*

Streptococcus gallolyticus, formerly classified as *S. bovis* biotype I, is an increasing cause of bacteremia and infective endocarditis in the elderly. Furthermore, several epidemiological studies have shown that the presence of this gram-positive coccus is consistently linked to colorectal cancer. We have studied the interaction of *S. gallolyticus* with the human coagulation and contact system. Activation of the contact system has two consequences: cleavage of high-molecular-weight kininogen (HK) resulting in release of the potent proinflammatory peptide bradykinin, and initiation of the intrinsic pathway of coagulation. *S. gallolyticus* was found to bind and activate the contact system at its surface, leading to a significant prolongation of the intrinsic coagulation time and also to the release of bradykinin, as shown by substrate and clotting assays, western blot analysis and ELISA. Importantly, our data indicate that *S. gallolyticus* can also escape and lyse human fibrin clots, characteristics, which aid the colonization and survival of bacteria infecting endocarditic vegetation. Binding and activation of contact factors is dependent on pili expression, supporting the role of adhesive pili as virulence factors in this strain.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

601/IIP
Regulation of human contact factors during streptococcal infection
J. Köhler*[1], B. Kreikemeyer[1], S. Oehmcke-Hecht[1]
[1]*University Medicine Rostock, Rostock, Germany*

The human contact system – also known as the intrinsic pathway of coagulation or kallikrein/kinin system – consists of four plasma proteins: factor XII (FXII), plasma kallikrein (PK), factor XI (FXI) and high-molecular-weight kininogen (HK). The contact system is activated as a response to bacterial infections, which supports it`s contribution to the early innate immune defense against bacteria. The mammalian liver responds to infection by a dramatic change in the synthesis of various plasma proteins. This phenomenon is known as hepatic acute-phase response and stimulated by several cytokines. Here we study the regulation of contact factors during an infection with *Streptococcus pyogenes*, an important human pathogen, by using the human hepatoma cell line HepG2. We show that HepG2 cells produce all contact factors. Infection of HepG2 cells with *S. pyogenes* increased production of contact factors, as shown by dot blot, western blot and ELISA. Our data indicate that human contact factors may act as positive acute phase proteins during an infection with *S. pyogenes*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

602/IIP
Real-time imaging of the bacillithiol redox potential in the human pathogen *Staphylococcus aureus* using a novel genetically encoded redox biosensor
V. L. Vu*[1], M. Harms[2], T. T. H. Nguyen[1], C. J. Hamilton[3], M. Müller[2], F. Hochgräfe[2], J. Pané-Farré[2], H. Antelmann[1]
[1]*Freie Universität Berlin, Berlin, Germany*
[2]*Ernst-Moritz-Arndt-University of Greifswald, Greifswald, Germany*
[3]*University of East Anglia, Norwich, United Kingdom*

Introduction: Bacillithiol (BSH) is the major low molecular weight thiol of *Staphyloccoccus aureus*. Under oxidative stress, BSH forms mixed disulfides with proteins, termed as S-bacillithiolation which can be reduced by glutaredoxin-like bacilliredoxins (Brx). In eukaryotes, glutaredoxin-fused roGFP2 redox biosensors have been applied for dynamic live-imaging of the glutathione redox potential.
Objectives: Here we have constructed the first genetically encoded bacilliredoxin-fused roGFP2 biosensor for dynamic live-imaging of the BSH redox potential in *S. aureus* (1).
Materials & Methods: The bacilliredoxin (Brx) of *S. aureus* was fused to roGFP2 and analysed for the response to different LMW thiol disulfides *in vitro*. Furthermore, Brx-roGFP2 was expressed in *S. aureus* COL and USA300 strains to monitor dynamic changes of the BSH redox potential *in vivo* using microplate reader measurements and confocal laser scanning microscopy.
Results: The Brx-roGFP2 biosensor showed a specific and rapid response to low levels bacillithiol disulphide (BSSB) *in vitro* which required the active-site Cys of Brx, but was unresponsive to cystine, GSSG or MSSM. Dynamic live-imaging revealed fast and dynamic responses of the Brx-roGFP2 biosensor inside *S. aureus* USA300 and COL strains under hypochlorite and H2O2 stress and constitutive oxidation of the probe in isogenic BSH-deficient mutants. Using confocal laser scanning microscopy, the changes in the BSH redox potential in *S. aureus* are confirmed in single cells. In phagocytosis assays with THP-1 macrophages, the biosensor was 87 % oxidized in *S. aureus* COL. However, no changes in the BSH redox potential were measured after treatment with different antibiotics classes indicating that antibiotics do not cause oxidative stress in *S. aureus*.
Conclusion: This novel Brx-roGFP2 biosensor can be used for live imaging of the BSH redox potential in *S. aureus* under oxidative stress, infection-like conditions and drug-treatments. We are currently analyzing the redox changes across human and zoonotic *S. aureus* isolates from different clonal complexes.

References
[1] Loi VV, Harms M, Müller M, Huyen NT, Hamilton CJ, Hochgräfe F, Pané-Farré J, Antelmann H. Real-Time Imaging of the Bacillithiol Redox Potential in the Human Pathogen *Staphylococcus aureus* Using a Genetically Encoded Bacilliredoxin-Fused Redox Biosensor. Antioxid Redox Signal. 2016. doi:10.1089/ars.2016.6733.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

603/IIP

The role of the novel hypochlorite-specific redox sensor HypS in *Mycobacterium smegmatis*

K. Klaper*[1], M. Hillion[1], M. Rossius[1], S. Gola[2], H. Antelmann[1]
[1]*Freie Universität Berlin , Institut für Biologie-Mikrobiologie, Berlin, Germany*
[2]*National Center for Biotechnology CNB - CSIC, Madrid, Spain*

Introduction: Mycothiol is the major low molecular weight thiol (LMW) produced in Actinomycetes. We have recently shown that MSH functions in protein S-mycothiolation in *Corynebacterium glutamicum* and *Mycobacterium smegmatis* under hypochlorite stress. In addition, the quantitative redox proteomics approach OxICAT revealed an increased oxidation level under NaOCl stress for more than 33% of all identified Cys residues.
Objectives: Among the highly oxidized proteins are several metabolic enzymes that are involved in energy metabolism, antioxidant functions and transcriptional regulation. We are interested to identify novel thiol-switches that are redox-controlled under oxidative stress conditions and confer protection against NaOCl.
Materials & Methods: Among the most highly oxidized NaOCl-sensitive proteins identified in the OxICAT dataset, an unknown MarR-family transcriptional regulator (renamed HypS) was further characterized using detailed genetic and biochemical studies. **Results:** HypS is conserved in the pathogen *M. tuberculosis* and encoded in the vicinity of genes for putative drug-efflux pumps. Thus, this novel MarR-type regulator could be important for the resistance to antibiotics or the defence to the host immune system. We have shown that HypS binds to its own promoter region and that its DNA-binding activity is reversibly inhibited by NaOCl treatment *in vitro*. DNA-binding assays using a Cys-Ser mutant under NaOCl stress showed that the single Cys of HypS is essential for redox-sensing *in vitro* since DNA-binding activity is not inhibited by NaOCl. However, treatment with H2O2 did not affect the DNA-binding activity of HypS *in vitro* suggesting that HypS senses specifically hypochlorite stress. First phenotype studies of a *hypS* deletion mutant revealed a NaOCl resistant phenotype indicating that HypS confers specific protection against NaOCl. Further studies are underway to identify the functions of the HypS-regulon members and to study redox regulation of HypS *in vivo*. **Conclusion:** Using quantitative redox proteomics we uncovered the novel redox-sensor HypS that is conserved in pathogenic Mycobacteria, highly oxidized under NaOCl stress *in vivo* and contributes to the defense against oxidative stress under infection-like conditions.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

604/IIP

Investigation of serum sFGL2 levels in Egyptian HCV and HCC patients

M. El-Mesery*[1], M. El-Mowafy[2], A. Elgaml[2], L. Farouk[1], S. Abed[3]
[1]*Faculty of Pharmacy, Mansoura University, Biochemistry, El-Mansoura, Egypt*
[2]*Mansoura University, Microbiology, Mansoura, Egypt*
[3]*Faculty of Medicine, Mansoura University, Department of Tropical Medicine, Mansoura, Egypt*

Infection with Hepatitis C virus (HCV) remains one of the serious human diseases worldwide especially in Egypt that can lead to cirrhosis or hepatocellular carcinoma (HCC). However, the exact molecular mechanism of HCC progress in HCV-infected patients remains unclear. Soluble fibrinogen-like 2 (sFGL2) is a modulator of immune response that is secreted by T cells and inhibits maturation of dendritic cells (DC) and T cell proliferation. In the current study, serum sFGL2 levels were analyzed by ELISA technique in chronic HCV-infected patients (n=30), chronic HCV-infected patients with HCC (n=30) and healthy control group (n=12). Moreover, serum levels of sFASL and IFN-γ were analyzed and correlated with sFGL2 levels. According to our results, serum sFGL2 levels were significantly elevated in all patients with chronic HCV infection. However, HCC patients showed lower sFGL2 levels than HCV-infected patients without HCC incidence. In addition, serum sFASL levels were significantly elevated in both HCV and HCC patients while serum IFN-γ levels were only elevated in HCC patients. Interestingly, sFGL2 correlated positively with serum total bilirubin level and negatively with serum levels of sFASL, IFN-γ and albumin in HCV and HCC patients. Thus, conclusively, elevated sFGL2 levels represent a novel diagnostic marker for chronic liver diseases that open future possibility of designing new treatment strategies for HCV infection targeting sFGL2 and its immune suppressive effect.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

605/IIP

***Helicobacter pylori* γ-glutamyl transferase favours recruitment of CD8 cells to the gastric mucosa**

R. Mejías Luque*[1], S. Wüstner[1], F. Anderl[1], C. Sachs[1], M. Vieth[2], M. Gerhard[1]
[1]*Technische Universität München, Institut für Medizinische Mikrobiologie, Immunologie und Hygiene, Munich, Germany*
[2]*Klinikum Bayreuth, Institut für Pathologie, Bayreuth, Germany*

Helicobacter pylori γ-glutamyl transferase (gGT) is a key bacterial virulence factor, described to be important for gastric colonization and related to the development of gastric pathology. Still, it is unclear how *H. pylori* gGT-specific effects on the hosts innate and adaptive immune responses contribute to bacterial colonization and pathology. To address this question, we infected mice with *H. pylori* gGT proficient or gGT deficient bacteria and performed an exhaustive analysis of the immune response towards the bacterium in mice showing comparable bacterial load. We observed that *H. pylori* gGT is important for initial colonization, but not essential, since bacteria lacking gGT can still colonize and persist, albeit at lower frequencies. When analysing immune responses, we observed that mice infected with gGT proficient bacteria showed a stronger pro-inflammatory innate immune response. Interestingly, mice infected with gGT deficient bacteria showed similar Th-cell responses but reduced IFNγ response when compared to mice infected with wild type bacteria. Reduced IFNγ levels could be linked to a differential recruitment of CD8+ cells to the stomach. Indeed, we observed that gGT activity of *H. pylori* human clinical isolates correlated with infiltration of CD8+ but not CD4+ cells to the stomach. Together, our data support a crucial role for *H. pylori* gGT in bacterial gastric colonization and indicate that gGT favours the recruitment of CD8+ to the gastric mucosa, which might play a yet unknown and important role during *H. pylori* infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

606/IIP

p38/MK-2 phosphorylates RIP-1 to limit cytotoxic RIP-1 signaling in bacteria-infected macrophages

J. Gropengießer[1], L. Novikova[1], H. Schimmeck[1], N. Czymmeck[1], K. Ruckdeschel*[1]
[1]*Institute for Medical Microbiology, Virology and Hygiene, UKE , Hamburg, Germany*

The enteropathogenic bacterium *Yersinia enterocolitica* engages the virulence protein YopP to deactivate pro-inflammatory NFkappaB and MAPK signaling pathways which triggers apoptosis in infected macrophages. The cellular signals that regulate onset of macrophage apoptosis are, however, yet poorly

defined. Using genetic and pharmacological approaches we found that RIP-1 and its kinase activity were required for efficient apoptosis induction by *Y. enterocolitica* YopP. We wondered about the mechanisms by which YopP may unleash the pro-apoptotic activity of RIP-1. It was revealed that YopP suppressed the phosphorylation of RIP-1 conferred by the p38/MK-2 pathway. This yet unrecognized MK-2-mediated RIP-1 phosphorylation loop was induced by TLR activation in macrophages and processed independently from TNFR1 stimulation. Importantly, the inhibition of the phosphorylation of RIP-1 by p38/MK-2 promoted bacteria-induced apoptosis when the IKK-beta kinase activity was impaired. This indicates that MK-2 phosphorylates RIP-1 to negatively regulate the RIP-1 pro-apoptotic activity. As YopP impairs the activation of p38/MK-2 as well as of IKK-beta, the cytoprotective phosphorylation of RIP-1 is lost upon *Yersinia* infection which facilitates RIP-1-dependent apoptosis. Thus, the concerted subversion of several pathways by YopP releases the pro-apoptotic activity of RIP-1 and converts RIP-1 to an inducer of macrophage apoptosis.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

607/IIP

YopM as a Bacteria-Derived Anti-Inflammatory Cell-Penetrating Peptide (CPP) for the Topical Treatment of Immune-Mediated Inflammatory Skin Diseases

A. Gossens*[1], M. Schneeweiß[2], M. Poceva[1], M. A. Schmidt[1], K. Loser[2], C. Rüter[1,2]
[1]*Institute of Infectiology, ZMBE , Münster, Germany*
[2]*Institute of Experimental Dermatology and Immunbiology of Skin, Department of Dermatology, University Hospital, Münster, Germany*

Psoriasis is one of the most common immune-mediated chronic inflammatory skin disorders affecting approximately 2-3% of the population. It is considered as an incurable, life-long skin condition that affects all races, age groups and both sexes. A common disadvantage of all currently available drugs for treating this cutaneous disease is that they need to be applied systemically. Previously, we identified and characterized the bacterial effector protein YopM of pathogenic *Yersinia* as a novel cell-penetrating peptide. Furthermore, once inside the cell, YopM is able to down-regulate the transcription of several pro-inflammatory cytokines such as TNF-α. These novel findings suggest a potential immunotherapeutic application.
To investigate whether YopM might be applicable as a topical therapeutic agent for the treatment of psoriasis, we utilized the murine imiquimod (IMQ)-induced psoriasis model. In this model, daily topical application of imiquimod (IMQ), a TLR7/8 ligand and potent immune activator, on mouse back skin induces and exacerbates psoriasis. Here, we applied rYopM either topically or subcutaneously for 5 consecutive days. Our results confirmed the self-delivering abilities of YopM across the cutaneous barrier for topically applied rYopM, and indicated a remarkable dampening of overt inflammatory reactions. Furthermore, truncated rYopM variants were generated via site-directed mutagenesis to determine domains required for the penetration of epithelial barriers and for its anti-inflammatory activity. We found out that a single nuclear localization signal might be sufficient for the translocation of YopM into the nucleus and fulfilling the anti-inflammatory properties. Therefore we aim to design a minimal construct, which is also capable of ameliorating the disease symptoms of Psoriasis *in vivo*. Candidates will then be tested in the IMQ-induced psoriasis model.
Taken together, our data indicate that epicutaneously applied YopM can penetrate across the cutaneous barrier in an IMQ-induced psoriasis mouse model and triggers remarkable anti-inflammatory effect. Therefore topical YopM treatment might be suitable for targeted therapy of immune-mediated inflammatory skin disorders.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

608/IIP

Nucleic acid dependent recognition of *S. pyogenes* by the innate immune system

I. Freund*[1], A. Hafner[1], V. Castiglia[2], P. Kovarik[2], T. Poth[3], A. Dalpke[1], T. Eigenbrod[1]
[1]*Medical Microbiology and Hygiene, Dept. of Infectious Diseases , Heidelberg, Germany*
[2]*Max F. Perutz Laboratories, Vienna Biocenter, Vienna, Austria*
[3]*Center for Model System and Comparative Pathology, Institute of Pathology, Heidelberg, Germany*

Introduction: Recognition and defense of pyogenic bacteria depends on the activation of innate immune cells by pattern recognition receptors (PRRs). Endosomal Toll-like receptors (TLRs) have been reported to recognize bacterial nucleic acids, whereby the murine TLR13 is unique among other PRRs. It senses a specific nucleotide motif of bacterial 23S rRNA, furthermore a single methylation within this sequence motif abolishes activation of TLR13. Yet, it remains to be shown whether nucleic acid detection contributes to the overall activation by whole bacteria during infection and if nucleic acid modifications alter bacterial recognition.
Objectives: *In vitro* cytokine production induced by *S. pyogenes* has previously been reported to be inhibited by bafilomycin A, an inhibitor of endosomal acidification. Based on these results, we wanted to further analyse Group A *Streptococcus* (GAS) infection with respect to dependence on nucleic acid recognition *in vitro* and *in vivo*.
Material & Methods: *In vitro* experiments: Bone marrow derived macrophages (BMDMs) from wild type C57BL/6 or Unc93B1 mutant mice, the latter defective in endosomal TLR signaling, were infected with various *S. pyogenes* strains at different multiplicity of infection (MOI). After 20 h, cell free supernatants were collected and cytokine production was measured by ELISA.
In vivo infection model: 8 to 12 weeks old female wildtype or Unc93B1 mutant mice were infected subcutaneously with 10^7 CFU *S. pyogenes* strain ATCC 12344 for 24 h. Proinflammatory cytokines in the serum were measured by ELISA and histological analysis of the skin lesion was performed.
Results: At lower MOIs, BMDMs showed a clear Unc93B1 dependency for production of proinflammatory cytokines in *in vitro* experiments. At higher MOIs, this effect vanished and BMDM of the Unc93B1 mutant reached similar cytokine levels compared to WT mice. Of note, different *S. pyogenes* strains differed in their relative dependency on Unc93B1. *In vivo*, however, Unc93B1 mice showed increased serum cytokine concentrations over a period of 24h. This was accompanied by increased tissue necrosis, elevated bacterial load and pronounced local inflammation at the site of infection. Local lesions in Unc93B1 mice were more severe than in WT mice.
Conclusion: Altogether, the *in vitro* data show a clear dependency on the nucleic acid recognizing endosomal TLRs with respect to cytokine secretion by macrophages. *In vivo* Unc93B1 mice were less efficient in clearing bacteria resulting in increased local infection and elevated systemic inflammation. The results argue for an important contribution of endosomal TLRs in defense of *S. pyogenes.*

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

609/IIP

NCAM1 (CD56) on human natural killer (NK) cells has a functional role in recognizing the fungal mold *Aspergillus fumigatus*

E. Weiß*[1], S. Ziegler[1], A. L. Schmitt[1], J. Schlegel[2], A. Burgert[2], U. Terpitz[2], M. Sauer[2], O. Kurzai[3], H. Einsele[1], J. Löffler[1]
[1]*University Hospital Wuerzburg, Medical Clinic and Policlinic ll, Wuerzburg, Germany*
[2]*Julius-Maximilians-University, Department of Biotechnology and Biophysics, Wuerzburg, Germany*
[3]*Hans-Knöll-Institute, Department of Fungal Septomics, Jena, Germany*

Introduction: The fungal mold *Aspergillus (A.) fumigatus* can cause invasive aspergillosis (IA) in immunocompromised patients suffering from hematological malignancies or after allogenic hematopoietic stem cell transplantation (HSCT). In a study from Stuehler et al. (2015) it was shown that HSCT patients with low natural killer (NK) cell counts show a more severe outcome of IA compared to patients with higher NK cell counts (Stuehler et al. 2015). Upon fungal confrontation, NK cells secrete IFN-γ and perforin and these soluble factors inhibit the fungal metabolism (Bouzani et al. 2011, Schmidt et al. 2011).
Objectives: We investigated the interaction of human blood NK cells with *A. fumigatus* and more precisely the role of the neural cell adhesion molecule 1 (NCAM1, CD56) during this interaction.
Material & Methods: NK cells were isolated from PBMCs of healthy donors, pre-stimulated with Pro-Leukine overnight and were then either stimulated with *A. fumigatus* germtubes (MOI 0.5) or with IL15 and Pro-Leukine (positive control) or left untreated for different time points. NK cell expression analyses of receptors and cytotoxic molecules were investigated by flow cytometry and RT-qPCR. Direct interaction of NK cells and *A. fumigatus* was analyzed by SEM, LSM and *d*STORM microscopy. By inhibiting the cytoskeleton prior to fungal co-cultivation, we investigated the role of actin filaments and microtubules in NK cell binding to the fungus. Functional analyses were performed by blocking NCAM1 with mAB GRP165 (kindly provided by Daniela Pende and Alessandro Moretta, Università di Genova, Italy) on the NK cell surface.
Results: Co-culture of NK cells with *A. fumigatus* induced the time dependent concentration of NCAM1 at the fungal interaction site. This re-localization was dependent on actin re-arrangements, direct contact, cultivation time and the presence of live *A. fumigatus* germ tubes. NCAM1 re-localization was not associated with apoptosis, internalization or shedding of NCAM1, nor did we see any changes on gene expression level. Fungal co-culture further induced NK cell activation by the up-regulation of the surface activation marker CD69. Blocking of NCAM1 diminished re-localization to the contact side and inhibited fungus- mediated NK cell activation, indicating a functional role of NCAM1.
Conclusion: Here we show that NK cells specifically use NCAM1 for direct binding to *A. fumigatus*. Furthermore, we postulate a functional role for NCAM1, since blocking of NCAM1 inhibited fungus mediated NK cell activation.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

610/IIP

The detrimental role of C5aR in meningococcal sepsis

J. Herrmann[1], M. Münstermann[1], L. Strobel[1], T. Woodruff[2], A. Klos[2,3], K. Johswich*[1]
[1]*University of Würzburg, IHM, Würzburg, Germany*
[2]*University of Queensland, School of Biomedical Sciences, Brisbane, Australia*
[3]*Hannover Medical School, Institute for Medical Microbiology and Hospital Hygiene, Hannover, Germany*

Introduction: The complement system is a critical innate determinant protecting the host against infectious agents. Its key functions are opsonisation, lysis via the membrane attack complex and initiation of inflammation. It is well established that the membrane attack complex is essential to control disseminated *N. meningitidis* infection. Yet, besides the assembly of the membrane attack complex, the inflammatory split fragment C5a is released during activation of the complement cascade in invasive meningococcemia. C5a activates its corresponding G-protein coupled receptor, C5aR, on multiple target cells. Especially granulocytes and macrophages are activated through C5aR and migrate to sites of infection in order to clear invading microorganisms. However, unbridled or sustained complement activation yields high C5a concentrations, which exacerbate inflammatory conditions and lead to paralysis of cellular effectors.
Objectives: Given the importance of complement activation during meningococcal sepsis, we hypothesized that besides the beneficial effects of complement lysis due to the membrane attack complex, there is also a concomitant detrimental effect mediated by the C5a/C5aR-axis. Hence, we speculated that C5aR-activation impacts disease pathophysiology.
Methods: As in vivo model for meningococcal sepsis, the mouse intraperitoneal infection model was used to compare WT and $C5aR^{-/-}$ genotypes. Clinical scoring was applied and survival rates, bacterial burden and plasma cytokines were assessed.
Results: Upon intraperitoneal challenge, elevated levels of complement anaphylatoxins C3a and C5a were detected in plasma of the mice. There was a striking correlation between bacterial burden and the plasma concentration of C5a, making a contribution of C5a to pathophysiology plausible. Indeed, when subjected to infection, $C5aR^{-/-}$ mice displayed ameliorated symptoms, significantly higher survival rates and lower levels of bacterial burden as well as cytokines in comparison to WT mice. Similarly, when C5aR was targeted by a peptide inhibitor prior to infection, WT mice demonstrated significant amelioration of sepsis symptoms and enhanced survival. Moreover, C5aR blockade was also effective when administered after sepsis induction.
Conclusion: The data indicate that activation of the C5a/C5aR-axis is detrimental during meningococcal sepsis. While assembly of the membrane attack complex is necessary to kill invasive meningococci, the production of C5a appears to be a downside to the strong complement activation during meningococcal sepsis that accounts for disease pathophysiology. Since pharmacologic inhibition of C5aR enhances sepsis outcome in the mouse model, C5aR may be an interesting target for adjuvant therapy of invasive meningococcal disease to ameliorate symptoms and enhance survival.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

611/IIP

Extracellular DNA Released by Non-Tuberculosis Mycobacteria Facilitates the Cytosolic Induction of IFN-β via the cGAS-STING-TBK1-IRF3/7 Dependent Pathway

N. Ruangkiattikul*[1], A. Nerlich[1], J. Spanier[2], N. Janze[1], K. Laarmann[1], U. Kalinke[2], S. Weiss[3], R. Goethe[1]
[1]*University of Veterinary Medicine Hannover, Institute for Microbiology, Hannover, Germany*
[2]*TWINCORE Centre for Experimental and Clinical Infection Research, Institute for Experimental Research, Hannover, Germany*
[3]*Helmholtz Centre for Infection Research, Department of Molecular Immunology, Braunschweig, Germany*

Question: Type I interferons (IFN-I) are important mediators in antimicrobial defense. IFN-I can be induced by cytosolic sensing of extracellular bacterial DNA (eDNA) and bacterial secondary messenger cyclic di-nucleotides. Non-tuberculosis mycobacteria (NTM) comprise of several important opportunistic and obligate pathogens those are able to infect and cause diseases in human and animals. Presently, the understanding of the role of IFN-I on the outcome of NTM infections still remains to be elucidated.

Methods: Primary murine bone marrow derived macrophages or RAW264.7 murine macrophage cell line were infected with pathogenic or non-pathogenic NTM. IFN-I response and its signaling pathway were determined by qRT-PCR, ELISA and immune blot analysis.
Results: Here we show that macrophages infected with pathogenic NTM of the *Mycobacterium avium* complex induced significantly lower IFN-β responses on the mRNA and protein level than infection with the non-pathogenic NTM *M. smegmatis*. To dissect the molecular mechanism of this phenomenon, we focus on the analysis of the ruminant pathogen *Mycobacterium avium* ssp. *paratuberculosis* (MAP) and *M. smegmatis*. We demonstrate that viability and phagocytic uptake of bacteria were required for IFN-β induction. Both mycobacteria induced IFN-β via the cGAS-STING-TBK1-IRF3/7 axis but were independent of neither TRIF, MyD88 signaling pathway nor ESX-1 secretion system. Notably, enhanced amounts of extracellular *M. smegmatis* DNA (eDNA) were found in the cytosol of infected macrophages as compared to MAP infection.
Conclusions: The results demonstrate that, in NTM infection, amount of mycobacterial eDNA released into the host cell cytosol is associated with the induction of IFN-I via cGAS-STING-TBK1-IRF3/7 pathway.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

612/IIP

Reduced activity against *A. fumigatus* by human NK cells from alloSCT patients *vs.* healthy donors

L. Marischen*[1], A. L. Schmitt[1], A. Englert[1], E. Weiss[1], K. Huenniger[2], O. Kurzai[2], H. Einsele[1], J. Loeffler[1]
[1]*University Hospital / Würzburg, II. Med. Clinic, Würzburg, Germany*
[2]*Leibniz Institute for Natural Product Research and Infection Biology, Fungal Septomics, Jena, Germany*

Introduction: *Aspergillus fumigatus* conidia are continually infiltrating the lungs of humans, where they are cleared by the immune system. While NK cells contribute just slightly in healthy individuals, they may become essential in alloSCT patients. Within the reconstitution process of the immune system after stem cell transplantation, NK cells appear early on and - potentially - are confronted with *A. fumigatus*. Since alloSCT patients are in severe danger to develop aspergillosis, the receptor expression pattern and functionality of NK cells may be critical for the outcome. Data generated in our study group showed an inducible release of cytokines such as CCL4/MIP-1β and an important role of CD56 in the direct contact between NK cells and *A. fumigatus*.
Objective: This pilot study was meant to characterize NK cells from alloSCT patients *vs.* healthy individuals with regard to their receptor expression and functionality. In the long-term, we search for a correlation between NK cell characteristics and the development of aspergillosis.
Materials and methods: PBMCs of healthy individuals and alloSCT patients were isolated and subsequently analysed for expression of surface markers such as CD56, CD69, CD7, CD2, NKp30 or NKG2D on NK cells by flow cytometry. In addition, freshly isolated NK cells were preincubated overnight with proleukin and then stimulated or not with IL-2/IL-15 or *A. fumigatus* germ tubes. The frequencies of receptor proteins on the cell surface were measured by FACS analysis, while cytokine release was determined by Multiplex ELISA.
Results: Subsequently to stimulation with *A. fumigatus*, NK cells from alloSCT patients showed a higher frequency of unbound CD56, but a lower CD69 expression on the cell surface. In general, isolated NK cells from alloSCT patients showed a reduced release of chemokines such as CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES, but a slightly elevated release of CXCL8/IL-8 compared to NK cells from healthy donors. Comparison of TNF-α or IL-1α release by NK cells from healthy donors *vs.* patients revealed just marginal differences, but IFN-γ release was detected in IL-2/IL-15 stimulated NK cells from healthy donors nearly exclusively.
Conclusion: NK cells contribute to the immune response towards *A. fumigatus* by binding to the fungus and releasing cytokines in order to activate and recruit other immune cells. In alloSCT patients, however, NK cells showed reduced binding capacity to *A. fumigatus*, a weaker activation status and a reduced release of cytokines after stimulation. Further experiments have to clarify, to what extend specific NK cell characteristics correlate with the development of aspergillosis.
This study was supported by DFG TR-124 / FungiNet (project A2).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

613/IIP

Impact of GPI-anchored proteins of the human-pathogenic fungus *Aspergillus fumigatus* on interaction with human innate immune cells

T. Eiffert[1], M. T. N. Hoang*[1], M. Föge*[1,2], S. Hartung[1,2], T. Heinekamp[3], A. A. Brakhage[3], M. von Lilienfeld-Toal[1,2]
[1]*Leibniz Institute for Natural Product Research and Infection Biology, Infections in Hematology and Oncology, Jena, Germany*
[2]*Clinic of Internal Medicine II, Hematology and Oncology, Jena, Germany*
[3]*Leibniz Institute for Natural Product Research and Infection Biology, Molecular and Applied Microbiology, Jena, Germany*

Introduction: The mould *Aspergillus fumigatus* is the most important airborne opportunistic fungal pathogen, able to infect immune compromised individuals such as chemotherapy patients or solid organ transplantation recipients, resulting in high mortality rates. Outgrowth of *A. fumigatus* conidia requires *de novo* synthesis and rearrangement of the fungal cell wall, which is orchestrated, among others, by GPI-anchored proteins.
Question: In total, *A. fumigatus* encodes for 125 putative GPI-anchored proteins, most of them with unknown function. In this study we aim to elucidate the function of those integral proteins by means of screening a GPI single knock-out strain library and evaluate their impact on host-pathogen interactions.
Methods: To this purpose, co-incubation experiments of selected GPI-mutant strains with freshly isolated human leukocytes are performed. Upon encounter of resting and pre-swollen conidia, we characterise the phagocytic activity of leukocytes, the expression pattern of key surface markers and the generation of reactive oxygen species by flow cytometry. Additionally, the survival rate of the tested strains is determined by plating assays.
Results: Co-incubation experiments of GPI-mutant strains with freshly isolated human neutrophilic granulocytes showed a decrease in phagocytosis rate of resting and pre-swollen conidia of Δ*cspA* (45 %) compared to the wild type after 4 h co-culture (65 %). In contrast, the strain Δ*cspA* led to a more rapid activation of leukocyte mobility, assayed by the significant reduction of CD62L on the immune cell surface. The increased activation also corresponds with a significant increase in ROS production by leukocytes upon stimulation with Δ*cspA*. Other GPI-mutant strains such as Δ*rodA*, Δ*utr2*, Δ*crf1* and Δ*mp2* also show differential phagocytosis and ROS production rates, although less pronounced. Ongoing survival plating assays are carried out to define the virulence potential of these mutant strains following encounter of leukocytes.
Conclusion: Put together, our study exemplifies that the success of *A. fumigatus* as an opportunistic human pathogen might also rely on the functional redundancy of cell wall-structuring GPI-anchored proteins. However, our screening will allow the identification of key GPI-anchored proteins in *A. fumigatus* and therefore help to improve prophylaxis and therapy in immune compromised leukaemia and hematopoietic stem cell transplantation patients.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

614/IIP
QUANTIM- Quantification of Innate Immune Function in Whole Blood Infection Assays
I. Leonhardt*[1], K. Hünniger[1], D. Thomas-Rüddel[2], T. Lehnert[3], M. T. Figge[3], O. Kurzai[1]
[1]*HKI, Fungal Septomics, Jena, Germany*
[2]*University Hospital Jena, Anesthesiology and Intensive Care Medicine, Jena, Germany*
[3]*HKI, Applied Systems Biology, Jena, Germany*

Introduction: Sepsis pathobiology is determined by fatal dysregulation of immune homeostasis. For successful implementation of immunomodulatory therapies it is necessary to classify sepsis patients in terms of their immune status. QUANTIM provides a new approach for individualized quantification of alteration in immune effector functions of septic patients. We"re utilizing a human whole blood model of infection with important sepsis pathogens combined with biomathematical modeling to quantify the global status of the innate immune response to infection (Hünniger *et al.*, PLOSCompBiol 2014). However, before the whole blood model is ready to be examined with as heterogeneous of a population as septic patients, it needs to be validated with a more homogeneous population.
Objectives: Within this pilot study we aimed to investigate the blood of patients that underwent cardiac surgery with extracorporeal circulation. This surgery is characterized by a time-defined and relatively homogeneous inflammatory stimulus. Furthermore, the use of the same patient at defined time points before and after surgery allows us to compare cellular functions at different stages of inflammation.
Methods: For this study, blood was obtained from patients before cardiac surgery (pre-operative), immediately after surgery (post-operative), and 1 day after admission to the intensive care. Whole blood infection was performed with two model pathogens, representing bacterial (*Staphylococcus aureus*) and fungal (*Candida albicans*) sepsis over a 4 h time course. Before infection, pathogens were stained with CellTracker™ Green (CT) and killed. Blood cell count was quantified via a hematological analyzer.
Results: Preliminary analysis showed a strong post-operative increase in white blood cell count, particularly for neutrophils. Interestingly, the increase in cell number resulted in a faster association of *C. albicans* and *S. aureus,* but did not correlate with a stronger activation in terms of neutrophils. Moreover, compared to monocytes and NK cells, neutrophils showed decreased level of activation in blood taken after surgery. Furthermore, the change in surface marker expression (CD16, CD10) indicated increased levels of immature granulocytes in blood taken after surgery. In ongoing experiments we will analyze blood of more patients and visualize differences in immune effector functions using advanced biomathematical modeling.
Conclusion: In conclusion, with QUANTIM we provide novel methods for individualized quantification of alterations in immune effector functions by using an *ex vivo* whole blood model of infection combined with advanced biomathematical quantification. Once optimized, analyzes of blood samples of sepsis patients and patients who have survived severe sepsis will follow in future studies.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

615/IIP
Cloning, expression and purification of *Pseudomonas aeruginos*a flagellin B molecule and determination of its vaccine potentials
N. Amirmozafari*[1], B. Behrouz[1], M. Mahdavi[2]
[1]*Iran University of Medical Sciences, School of Medicine, Microbiology Dept., Tehran, Islamic Republic Of Iran*
[2]*Pasteur Institute of Iran, Tehran, Islamic Republic Of Iran*

Pseudomonas aeruginosa is an opportunistic pathogen that causes serious infections in immunocompromised hosts including cystic fibrosis, cancer, and severely burned patients. Proliferation of P. aeruginosa within burned wounds leads to septicemia which is often associated with a high degree of mortality. The single polar flagellum on this bacterium is essential for motility, chemotaxis, invasiveness, adhesion, and activation of host inflammatory responses. In this investigation, the protective efficacy of recombinant type B flagellin was tested by active and passive immunization in burned mice infected with lethal dose of P. aeruginosa. The immunogenicity and efficacy of the type B flagellin as a vaccine candidate was evaluated in a burned mouse model.
The type B flagellin gene from the PA01 strain was amplified by PCR and cloned into pET-28a expression vector. After over-expression in E. coli strain BL21, the recombinant flagellin was purified through dissolution of inclusion bodies in 8.0 M urea, absorption to Ni-NTA resin, and final elution by imidazole solution. The immune reactivity was confirmed by western blot. Active immunizations were carried out by injection of 10 µg of the recombinant protein with and/or without alum adjuvant in different groups of mice. Titers of IgG in pooled sera were evaluated by ELISA. Opsonophagocytosis assays were performed in order to measure functional antibody activity. Lymphocytes were cultured in the presence of the antigen and 72 hr culture supernatants of the activated macrophages were analyzed for cytokine profiles (IL-4, IFN-ϒ, and IL-17) by ELISA. The prophylactic and therapeutic effects of rabbit polyclonal IgG raised against the recombinant protein were tested in murine burned mice.
The antibodies obtained from immunized mice and rabbits showed significant opsonophagocytic killing activity. Immunization led to significant production of IL-4, IFN-ϒ, and IL-17 by antigen stimulated lymphocytes. Pre-immunized burned and P.aeruginosa infected mice showed significant enhanced survival rates compared to the control group. Survival was correlated with a reduced systemic microbial load.
These results demonstrate that immunization with the recombinant type B flagellin vaccine candidate in mice increased the levels of humoral and cellular immune responses which could lead to higher protection against P. aeruginosa burn infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

616/IIP
Investigation of the Immunomodulatory effect of Berberis vulgaris on core-pulsed dendritic cell vaccine
M. Aziz*[1], D. Ghareeb[1]
[1]*Faculty of Science, Alexandria University, Biochemistry, Alexandria, Egypt*

Background: Virus-induced dendritic cells (DCs) functional deficiency leads to sub-optimal initiation of adaptive immune responses and consequently chronic infection establishment. The present study reports an advanced hepatitis C virus (HCV) therapeutic vaccine model based on In vivo enrichment of DCs with barberry ethanolic crude extract (BCE) then pulsing them with HCV core protein.
Methods

DCs were enriched by BCE intravenous injection in BALB/c mice. Vaccine efficiency was assessed by flow cytometric analysis of splenocytes of immunized mice, cytokine profiling, cytotoxic T lymphocyte assay, and humoral immune response assessment.
Results: There was no significant difference in surface phenotypic characterization of splenocytes from mice immunized with non-BCE-enriched-core-pulsed DCs (iDcs-core) compared to those from mice injected with RPMI-1640 medium. However, splenocytes from mice immunized with BCE-enriched-core-pulsed DCs showed 197 % increase in CD16+ population, 33 % increase in $MHCII^+$ population, and 43 % decrease in $CD3^+$ population. In iDCs-core group, 57.9 % greater anti-core cytotoxic T lymphocyte activity, up-regulation in interferon gamma and interleukin (IL) -12 expression, and down-regulation in IL-4 and IL-10 were recorded. Moreover, sustained specific anti-core antibodies were detected only in sera of the same group.
Conclusions: Results indicate that BCE-enriched-core-transduced DCs may serve as a new model for immunotherapy of HCV chronic infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

617/IIV
***Candida albicans* evades phagocytosis and killing in a host dependent process**
K. Hünniger*[1], T. Lehnert[1], M. T. Figge[1], O. Kurzai[1]
[1]*HKI Jena, Fungal Septomics, Jena, Germany*

Introduction: *Candida albicans* is the major cause of hospital acquired systemic fungal infections which, due to high mortality rates, have become an increasingly prominent clinical problem. In the present study, we used a human whole-blood model to analyze the interplay of *C. albicans* with host immunity during the initial phase of infection. Our results identified a fraction of *C. albicans* cells that remained extracellular and some fungi also survived throughout the experiment.
Objectives: This study is dedicated towards the elucidation of a novel immune escape mechanism that enables the persistence of viable extracellular *C. albicans* cells in human blood for prolonged time periods.
Methods: Human peripheral blood or primary immune cells isolated from whole blood were confronted with *C. albicans* and incubated for different time points at 37°C. Innate immune activation, immune cell-fungal interaction and deposition of host factors like complement components on the fungal surface were followed either by flow cytometry or specifically during confrontation with isolated immune cells on a single cell level by confocal microscopy.
Results: Simulation of experimental data by our virtual infection model predicted that almost all *C. albicans* cells, which remained alive during prolonged human whole blood infection had developed resistance against phagocytosis. Furthermore, biomathematical modeling suggested that immune escape is strongly related to neutrophil activity and predicted enhanced numbers of extracellular *C. albicans cells* in neutropenic patients - which fits to the clinical observation that dissemination of *C. albicans* bloodstream infection is more common in this patient cohort. Based on identification of complement receptor 3 (CR3) as the major uptake receptor for *C. albicans* in blood, we analyzed opsonization of fungi during infection. Extracellular *C. albicans* rapidly bound C3b/iC3b, but opsonization steadily decreased over time. Following two hours of whole blood infection no C3b/iC3b signal was detectable on *C. albicans* surface anymore. Surprisingly, this was independent of fungal virulence traits or even viability of the fungal cells, suggesting host mediated remodeling of the fungal surface. Using confrontation assays with isolated cells like primary PMN, monocytes, NK cells or platelets, we got the evidence that PMN (and to lesser extent monocytes) mediate this remodeling independent of fungal activity. Currently, we are systematically analysing the molecular mechanisms enabling host mediated cell wall remodeling using established *ex vivo* infection models and proteomic analyses.
Conclusion: Taken together, immune escape in an extracellular population of fungal cells may be the most favourable explanation for long-term survival of *C. albicans* in human blood and may explain the high proportion of dissemination in *C. albicans* bloodstream infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

618/IIP
Molecular basis for Rho-family GTPase discrimination by a bacterial virulence factor
N. Dietz*[1], A. Harms[1], I. Sorg[1], G. Mas[1], A. Goepfert[1], S. Hiller[1], T. Schirmer[1], C. Dehio[1]
[1]*University of Basel, Biozentrum, Basel, Switzerland*

Small GTPases of the Ras-superfamily are molecular switches that control fundamental cellular functions in eukaryotes by cycling between GTP-bound "on" and GDP-bound "off" conformational states of their switch regions 1 (Sw1) and 2 (Sw2). The Ras-homology (Rho) protein-family is defined by the variable, 13 residues long Rho-insert close to the C-terminus that is implicated in wiring Rho-family GTPases to their specific biological functions. Additionally, the otherwise invariable TKxD nucleotide-binding motif is changed to TQxD in a subset of Rho-family GTPases. Rho-family GTPases function as signalling hubs and regulate cytoskeletal rearrangements, cell motility, and the production of reactive oxygen species.
A plethora of bacterial virulence factors—either effector proteins that are translocated into the host cell by dedicated secretion systems or secreted toxins that autonomously enter host cells—stimulate, attenuate or destroy the intrinsic GTPase activity of Rho-family GTPases and several strategies to manipulate GTPase functions have been described, such as mimicking guanine nucleotide exchange factor (GEF) or GTPase-activating protein (GAP) function, or covalent modification of residues in the Sw1 or Sw2 regions. However, the structural basis for selective targeting of Rho-family GTPase subsets has so far remained unknown.
Among bacterial virulence factors that covalently modify Rho-family GTPases, FIC (filamentation induced by cyclic AMP) domain proteins represent an increasingly recognized enzyme family that typically catalyses the covalent transfer of AMP to target hydroxyl side-chains. Prominent examples are the FIC domains of IbpA from *Histophilus somnii* and VopS from *Vibrio parahaemolyticus*, which both target a wide range of Rho-family GTPases and AMPylate (adenylylate) a conserved tyrosine or threonine residue, respectively, in Sw1. Both modifications result in abrogation of downstream signalling causing collapse of the cytoskeleton of the host cell and subsequent cell death. Here, we show that the FIC domain of *Bartonella* effector protein (Bep) 1 from *Bartonella rochalimae* AMPylates the same Sw1 tyrosine residue as IbpA, while the target spectrum is strictly limited to the Rac-subfamily. Employing a combination of biochemistry, crystallography, nuclear magnetic resonance (NMR) spectroscopy and mutational analysis, we identify the structural determinants of this remarkable target selectivity. Our findings further provide a rationale for altering Bep1 target selectivity in order to craft novel tools with surgical precision for dissecting Rho-family GTPase activities.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

619/IIP

Myeloperoxidase is a major factor in the breakdown of the thiol reduction potential in phagocytized bacteria

A. Degrossoli[1], J. Schneider[1], K. Xie[1], A. Müller[1], V. Bader[2], K. Winklhofer[2], L. Leichert*[1]
[1]*Ruhr-Universität Bochum, Biochemie der Mikroorganismen, Bochum, Germany*
[2]*Ruhr-Universität Bochum, Molekulare Zellbiologie, Bochum, Germany*

Phagocytic immune cells kill pathogens in the phagolysosomal compartment with a cocktail of antimicrobial agents. Chief among them are reactive species produced in the so-called oxidative burst. Here we show that bacteria exposed to a neutrophil-like cell line experience a rapid and massive oxidation of cytosolic thiols. Using roGFP2 based fusion probes, we could show that this massive breakdown of the cellular thiol reduction potential was dependent on phagocytosis, presence of NADPH oxidase and ultimately myeloperoxidase. The hydrogen peroxide sensitive roGFP2-Orp1 fusion protein was rapidly oxidized in bacterial cells phagocytized by immune cells. Inhibition of phagocytosis in immune cells by cytocalasin D led to abrogation of probe oxidation in bacteria. Similarly, probe oxidation was absent in cells lacking $gp91^{phox}$, the catalytic subunit of NADPH oxidase NOX2. Interestingly, the redox-mediated fluorescence change in bacteria expressing a glutathione specific Grx1-roGFP2 fusion protein or an unfused roGFP2 showed highly similar reaction kinetics to the ones observed with roGFP2-Orp1, under all conditions tested. We therefore suspected that either high oxidant concentration or the presence of a highly reactive species in the phagolysosome could essentially bypass the fusion proteins Grx1 or Orp1 and directly and rapidly oxidize the redox sensing cysteines in roGFP2. We recently observed such an indiscriminate oxidation of roGFP2-based fusion probes by HOCl with fast kinetics *in vitro*. Abating HOCl-production in immune cells with a myeloperoxidase inhibitor significantly attenuated the oxidation of all three probes in bacteria. Probing the oxidation state of cysteines of bacterial proteins that are known to be targeted by HOCl further substantiated the major role of Myeloperoxidase in the disturbance of the bacterial thiol redox state.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

620/IIP

Human plasma factor H-related protein 1 (FHR1) binds to *C. albicans* and acts pro-inflammatory in monocytes

S. Irmscher*[1], S. Hidalgo Vico[1], B. Hube[1], C. Skerka[1]
[1]*Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut , Jena, Germany*

The complement system is the first line of defence against microbial infections and once activated is tightly regulated by membrane bound and soluble regulators. FHR1, which is known to inhibit the C5 convertase as well as the MAC assembly in fluid phase is recruited by the pathogenic fungus *C. albicans*. However, bound to surfaces FHR1 acts as complement activator and stimulates the inflammasome in monocytes to release the pro-inflammatory cytokine IL-1b. In addition anti-inflammatory IL-10 is reduced. Monocyte regulation by FHR1 is dependent on normal human serum but independent of complement activation. How FHR1 mediates the pro-inflammatory effect in monocytes and why *C. albicans* recruits the protein to the surface is still unclear.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Clinical Microbiology and Infectious Diseases (StAG KM)

621/KMP

Neuroborreliosis mimicking Acute Encephalopathy: The use of CXCL13 as a biomarker in CNS manifestations of Lyme-Borreliosis

M. Karrasch*[1], V. Fingerle[2], K. Boden[3], A. Darr[4], M. Baier[5], E. Straube[5], I. Nenadic[6]
[1]*Institute of Medical Microbiology, Jena University Hospital, Jena, Germany*
[2]*National Reference Center for Borrelia, Bavarian Health and Food Safety Authority, Oberschleißheim, Germany*
[3]*Institute of Clinical Chemistry and Laboratory Medicine, Jena University Hospital, Jena, Germany*
[4]*Department of Nuclear Medicine, Jena University Hospital, Jena, Germany*
[5]*Institute of Medical Microbiology, Jena, Germany*
[6]*Department of Psychiatry and Psychotherapy, Jena University Hospital, Jena, Germany*

Introduction: We report an unusual case with an acute onset of psychiatric episodes in whom the diagnosis neuroborreliosis (NB) could be established.
Material & Methods: Screening immunoassays were used for specific Borrelia burgdorferi IgG/-IgM antibodies (Enzygnost, Siemens Healthcare, Marburg, Germany). RecomLine Borrelia (Mikrogen, Neuried, Germany) was used for antibody detection against all immunopathogenic Borrelia genospecies in order to confirm EIA results. Chemokine CXCL13 measurement (R&D Systems, Minneapolis, USA) and cytology analysis was done in CSF. Detection of B. burgdorferi s.l. from CSF was done by culture and RT-PCR targeting p41 and ospA. PCR targeting the hbb with melting curve analysis and MLST using eight housekeeping genes was performed to establish the B. burgdorferi spezies.
Results: Serology (ELISA) showed a Borrelia-IgG value of 480 U/ml and -IgM of 0.5 U/ml. CSF ELISA Borrelia-IgM was at 1.668 U/ml. OSPC and VlsE were detected in Borrelia-specific IgG serum blot; p41 was detected in Borrelia-IgM CSF blot. Borrelia-IgG antibody index (AI) was at 69.80, IgM AI at 19.4. TPPA and FSME testing was negative. CSF cytology showed highly elevated total protein and total cell number (260 cells/µl) with lymphocytic cell reaction, highly elevated lactate, severe blood-brain barrier dysfunction and intrathecal IgG- and IgM-synthesis. Both, ospA and p41 RT-PCR were positive. Borrelia genospecies identification failed. CXCL13 was 39.000 pg/ml at time of NB diagnosis (>250 pg/ml positive) and dropped to 2972 pg/ml and 120 pg/ml, respectively.
Discussion: NB is known to cause cognitive dysfunction in adults. Diagnosis of acute NB was fulfilled by clinical symptoms, CSF pleocytosis, and intrathecal production of Borrelia-specific antibodies. CSF CXCL13 was used as an additional non-Borrelia specific determinant in early NB. There was a positive correlation between CXCL13 titer decline, reduction of CSF pleocytosis and improvement of clinical symptoms under successful antibiotic therapy.
Conclusion: Patients suffering from acute onset psychiatric disorders should be screened for NB. Although CXCL13 is not yet validated as a routine diagnostic tool, CSF CXCL13 may be another option to increase sensitivity and accuracy in diagnosing NB, next to CSF lymphocytic pleocytosis.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

622/KMP

Determination quantitative composition of the microbiota in parietal intestinal surface in rats by PCR real-time

Y. Bukina*[1], A. Kamyshny[1]
[1]*Zaporozhye State Medical University, Microbiology, Virusology and Immunology, Zaporozhye, Ukraine*

Violation of the intestinal microbiota lead to dysbiotic symptoms and increased susceptibility to bacterial agents.
The purpose and task of research: to analyze the change of quantative and species composition of microflora in the parietal intestine surface of rats after oral administration of *vancomycin* and *Salmonella enteritidis*, *Salmonella typhimurium*.
Research methods: The material used for PCR studies parietal contents of the parietal intestine, resulting in the opening of 40 rats "Wistar", divided into groups: I - *control group*; II - *Vancomycin*; III - *S.enteritidis*; IV - *S.typhimurium*. When quantifying the specific areas of bacterial DNA kits of reagents used "DNA Express" format "FLUOROPOL-RT" ("Liteh", Russia). The evaluation results of amplification was performed using program Bio-Rad CFX Manager 3.0.
Results: In groups II, III, IV reduces the number of *E.coli* in 8, 4, 120 times; *Bacteroides spp.* in 376, 55, 32 times; *E.faecalis, E.faecium* in 394 3, 1311 times; *Peptostreptococcus anaerobius* in 303, 453 and 300 times; *P.aeruginosa* times in 3813 (III); and increasing the number of *Salmonella spp.* in 10, 3728, and 1393 times; *Shigella spp.* in 17, 29, 98 times; *Proteus spp.* in 25 (II) and several thousand times (III, IV); *Enterobacter spp., Klebsiella spp.* increased in 18 times (II) and decreased in 434, 358 times (III, IV group) ($p \leq 0,05$).
Conclusions: The data show that the administration of *vancomycin* and *S.enteritidis, S.typhimurium* content in the wall of intestine reduces the number of *Bacteroides*, *Enterococci*, *Peptostreptococci*, *Pseudomonas* and increasing the number of *Enterobacteria*, *Proteus*, *Klebsiella*, *Salmonella* and *Shigella*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

623/KMP

Changing the Number of the Wall of the Intestinal Microflora in Rats when a administered Salmonella by PCR Real-Time

Y. Bukina*[1]
[1]*Zaporozhye State Medical University, Microbiology,virusology and immunology, Zaporozhye, Ukraine*

Violation of the intestinal microbiota lead to dysbiotic symptoms and increased susceptibility to bacterial agents.
The purpose and task of research: to analyze the change of quantative and species composition of microflora in the parietal intestine surface of rats after oral administration of *vancomycin* and *Salmonella enteritidis*, *Salmonella typhimurium*.
Research methods: The material used for PCR studies parietal contents of the parietal intestine, resulting in the opening of 40 rats "Wistar", divided into groups: I - *control group*; II - *Vancomycin*; III - *S.enteritidis*; IV - *S.typhimurium*. When quantifying the specific areas of bacterial DNA kits of reagents used "DNA Express" format "FLUOROPOL-RT" ("Liteh", Russia). The evaluation results of amplification was performed using program Bio-Rad CFX Manager 3.0.
Results: In groups II, III, IV reduces the number of *E.coli* in 8, 4, 120 times; *Bacteroides spp.* in 376, 55, 32 times; *E.faecalis, E.faecium* in 394 3, 1311 times; *Peptostreptococcus anaerobius* in 303, 453 and 300 times; *P.aeruginosa* times in 3813 (III); and increasing the number of *Salmonella spp.* in 10, 3728, and 1393 times; *Shigella spp.* in 17, 29, 98 times; *Proteus spp.* in 25 (II) and several thousand times (III, IV); *Enterobacter spp., Klebsiella spp.* increased in 18 times (II) and decreased in 434, 358 times (III, IV group) ($p \leq 0,05$).
Conclusions: The data show that the administration of *vancomycin* and *S.enteritidis, S.typhimurium* content in the wall of intestine reduces the number of *Bacteroides*, *Enterococci*, *Peptostreptococci*, *Pseudomonas* and increasing the number of *Enterobacteria*, *Proteus*, *Klebsiella*, *Salmonella* and *Shigella*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

624/KMP

Macrolide and quinolone resistance-related mutations in *Mycoplasma genitalium* strains in Dresden, Germany

R. Dumke*[1], A. Thürmer[1], E. Jacobs[1]
[1]*TU Dresden, Institut für Medizinische Mikrobiologie und Hygiene, Dresden, Germany*

Mycoplasma genitalium (M.g.) is a sexually transmitted bacterium associated with nongonococcal urethritis in men and with cervicitis and pelvic inflammatory disease in women. Cultivation of the fastidious mycoplasmas is difficult and detection of infections based on molecular methods mainly. According to the intrinsic resistance of the cell wall-less bacteria to betalactam antibiotics, use of macrolides (azithromycin) has been considered as first-line treatment for many years. Recently, an increasing number of treatment failures were reported. Macrolide resistance in M.g. results from mutations at positions 2058 and/or 2059 (*Escherichia coli* numbering) in domain V of 23S rRNA. A single dose of azithromycin can cause the selection of mutants harboring these sequence changes with the consequence of a high level of resistance. Fluoroquinolones have been found successful in cases with azithromycin treatment failure and are widely used as second-line antibiotics. Unfortunately, occurrence of point mutations in the quinolone resistance-determining region (QRDR) of the topoisomerase IV gene (ParC) suggests the world-wide emergence of strains with decreased susceptibility to fluoroquinolones. Knowledge of resistance rates of M.g. strains in Germany is limited. In the present study, we investigated specimens sent between September 2014 and November 2017 to our laboratory (n=553). M.g. was detected in 41 patients by using a commercial real-time PCR (TIBMolbiol). Partial sequencing of 23S rRNA showed macrolide resistance-associated mutations in 44% of strains (15x A2059G; 2x A2058G; 1x A2058T). Furthermore, a T2068C transition was found for the first time. Testing of phenotypic macrolide susceptibility of strain is needed to evaluate the importance of this mutation for resistance. In addition, three patients harbor M.g. strains with mutations of QRDR of ParC (aa81: Gly to Cys; aa83: Ser to Arg or Ile). In parallel, one of these strains is macrolide-resistant limiting the treatment options strongly. The findings of the study confirm a relatively high rate of macrolide resistance among local M.g. strains in Germany. Additionally, few strains were found showing mutations which are suggested as related to quinolone resistance. Beside the fact that further investigations are necessary to collect more nation-wide data of resistance, the results emphasize a continuous monitoring of this agent of sexually transmitted infections of humans.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

625/KMP
Analysis of a novel anti-chlamydial compound in active and persistent chlamydial cell culture infection models
M. Brunke*[1], A. Klöckner[1], K. Shima[2], J. Rupp[2], H. Bühl[1], J. Reuter[1], H. G. Sahl[1], F. Götz[3], B. Henrichfreise[1]
[1]*University of Bonn, Institute for pharmaceutical Microbiology, Bonn, Germany*
[2]*University of Lübeck, Lübeck, Germany*
[3]*University of Tübingen, Tübingen, Germany*

Chlamydiae undergo a biphasic developmental cycle or enter a stage of persistence to evade stressful conditions *in vitro* and the role of persisting, viable but non-dividing chlamydial cells in the development of chronic diseases in patients is matter of debate in the field.
We identified a compound that clears active infections with *C. trachomatis* in cell culture at a concentration of 2 mg/L - without detectable toxic effects on eukaryotic host cells as monitored by cell viability assays - and present data from IFN-gamma and penicillin induced persistence models. Of interest, the compound impairs persisting chlamydial cells in both persistence models.
Our data help to achieve insight into the mode of action of the compound and might contribute to the design of anti-infective strategies against active and persistent infections with the non-model organism *Chlamydia*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

626/KMP
Isolation, Identification and Characterization of *Vibrio* spp. from suspected human cases.
M. T. Rahman*[1], M. T. Hossain[1], F. Zereen[1]
[1]*Bangladesh Agricultural University, Microbiology and Hygiene, Mymensingh, Bangladesh*

Vibrio spp. particularly *V.cholerae* has been recognized as one of the most important cause of diarrhea in human worldwide. Hence, the research work was undertaken with a view to isolate, identifies and characterizes *Vibrio* spp. For these purpose samples (fresh diarrheal stool, rectal swab) were collected from suspected to be infected human patient of Surjo Kanto hospital, Mymensingh. A total of 25 samples were subjected to bacterial isolation and identification by using cultural morphological and biochemical techniques. Among these samples 7 were suspected for the presence of *Vibrio* spp. Isolates were confirmed as *Vibrio* spp.by PCR with primers specific for *Vibrio groEL* gene. Furthermore, the isolated *Vibrio* spp.were characterized by antimicrobial susceptibility testing. The antibiotic sensitivity test with market available common antibiotics revealed that, the isolated bacteria showed sensitivity to Chloramphenicol, Ciprofloxacin, Erythromycin, Azithromycin and resistant to Streptomycin and Oxacillin.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

627/KMP
Investigating the metabolism of uropathogenic *E. coli* during infection
S. Mortensen*[1]
[1]*University of Copenhagen, Department of Veterinary Disease Biology, Frederiksberg C, Denmark*

Most existing antibiotics inhibit enzymatic processes that are involved in polymer synthesis outside the metabolism (synthesis of proteins, RNA, DNA or peptidoglycan), and only sulphonamides and trimethoprim target biosynthesis of an essential biomass component (tetrahydrofolic acid). The reason is that bacteria have a high degree of redundancy in their enzyme systems (different systems can provide the same essential metabolites), and because we know very little on which biomass precursors they scavenge from their host during infection, and thus circumvent the need for de novo biosynthesis. It has recently been suggested and preliminarily shown that antibiotics that target two non-essential, but redundant metabolic enzymes, can be used to stop infections. It is not feasible to identify redundant enzymes by traditional genetic screens, since it will require combined mutagenesis of all known metabolic enzyme-encoding genes. Therefore, one needs to use a metabolic modelling approach whereby computer simulations produce *in silico* predictions of which pathways/networks within the bacteria that are redundant, given a particular growth condition. In this study, we focus on the metabolism of uropathogenic *E. coli* (UPEC) during urinary tract infections (UTIs). UPEC is the most common causative agent of UTI and thus a major reason for use of antibiotics. We have characterized the metabolism by a proteomic approach for UPEC growing in test tube in minimal defined media, in human urine, in human bladder carcinoma cells and while infecting an avian model of salphingitis. The aim is to construct a metabolic model for UPEC, and by feeding the model with the proteome data, we will be capable of providing an accurate simulation of the metabolism in the different growth conditions. This will not only allow us to predict essential metabolic enzymes, but also to predict the most likely redundant enzymes by removing all possible combinations of two metabolic enzymes from the model and see how that affects the metabolism. At the conference, we will highlight the features of the metabolic model by possible comparisons to other models, and finally, give examples of redundant pairs of metabolic enzymes in UPEC.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

628/KMP
Clonal relationship among isolates of *Bacillus* sp isolated from tobacco and lung cancer biopsies
A. Merlos[1], E. Parra[1], E. Armengol[1], S. Martí[1], T. Vinuesa[1], M. Vinas*[1], J. M. Sierra[1]
[1]*University of Barcelona, Pathology & Experimental Therapeutics, Hospitalet, Spain*

Bacillus species are associated with bacteremia/septicemia, endocarditis, meningitis, and infections of wounds, the ears, eyes, respiratory, urinary, and gastrointestinal tract. Interestingly, *Bacillus* species have also been reported to be found in tobacco cigarettes and in lung cancer biopsies. Relationship between the strains isolated from commercial tobacco and lung cancer biopsies has been explored.
Several brands of commercial tobacco were microbiologically examined by cutting 1 cm of each cigarette and transferred to 5 ml of saline. After 5 min of soaking, suspensions were treated in ultrasonic water bath for 30 s and vortexed for 2 min. Finally, samples were centrifuged at 3000 rpm for 1 min. 100 µl of the supernatant were cultured in different media: TSA, blood-agar, Sabouraud-Chloramphenicol and thioglycolate broth. Similarly, for lung cancer biopsies, samples were introduced into 1.5 ml tubes with 1 ml of Ringer prokaryotes medium immediately after the tissue operation -transportation took place as fast as possible-. From each biopsy, one half was frozen at -80°C, and the other was mechanically homogenised and cultured in the same media and under the same conditions as the ones aforementioned.
Bacterial identification was carried out by performing biochemical tests and 16S gene sequence. PFGE was performed as previously described, and restriction enzyme notI or sfiI was used depending on the *Bacillus* species. Interpretation of the PFGE patterns was made following Tenover criteria.
A total of 55 strains of *Bacillus* strains were recovered, 40 strains were isolated from tobacco and 15 strains were isolated from lung cancer biopsies. The strains belonged to 7 different species of

Bacillus, 8 strains of *B. amyloliquefaciens* , 8 strains of *B. clausii*, 4 strains of *B. cereus* and 1 strain of *Oceanobacillus chungangensis* (All of them isolated from tobacco), and 15 strains of *B.subtilis* (9 from tobacco and 6 from lung cancer biopsies), 14 *B. pumilus* (8 from tobacco and 6 from lung cancer biopsies), 5 strains of *B. liqueniformis* (2 from tobacco and 3 from lung cancer biopsies). These strains were characterized as toxin producers and involved in ling cancer (Merlos et al., 2015)
Analysis of PGFE patterns of each species isolated in both (tobacco and lung cancer biopsies) showed a clonal relationship among the strains isolated from lung cancer biopsies and no relationship with those strains isolated from commercial tobacco. Similar results were obtained for the 3 studied species.
These results suggested that only some specific clones of *B. subtilis*, *B. pumilus* and *B. licheniformis* are able to colonize and/or infect lung tissues.

References
[1] Merlos A, Rodríguez P, Bárcena-Uribarri I, Winterhalter M, Benz R, Vinuesa T, Moya JA, Viñas M. Toxins Secreted by *Bacillus* Isolated from Lung Adenocarcinomas Favor the Penetration of Toxic Substances. Front Microbiol. 2015 Nov 23;6:1301

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

629/KMP
Identification of Coxiella burnetii targets for development of new Q fever diagnostics in ruminants
C. Gerlach*[1], G. Flores-Ramirez[2], L. Skultety[2], K. Henning[1], H. Neubauer[1], K. Mertens[1]
[1]*Friedrich Loeffler Institute, Institute of Bacterial Infections and Zoonoses, Jena, Germany*
[2]*Slovak Academy of Sciences, Bratislava, Slovakia*

Coxiella burnetii is the etiological agent of the almost worldwide distributed zoonosis Q fever, which represents in human in 40% of cases as acute self-limiting disease and can progress to chronic life-threatening stage in 1-5% of patients. Ruminants are considered as the main reservoir for human infections. The often subclinical infection in ruminants and suboptimal sensitivity of current diagnostics hamper the identification of infected animals. Current serological diagnostics are based on whole cell antigen and lack well defined and reproducible epitopes. The aim of this study is to develop a highly sensitive and specific serological method for Q fever detection. Therefore *in silico* predicted B cell epitopes of published as well as experimentally identified immunogenic proteins will be selected for amino acid sequence identity between *C. burnetii* isolates and for sufficient polymorphism to related pathogens.
From literature 52 *C. burnetii* immunogenic proteins have been chosen based on the number of publications and analyzed sera. Of all proteins 23% are within the cytoplasm, 15% in the outer membrane, 10% in the cytoplasmic membrane, 6% exported and 44% are of unknown localization. Interestingly, most antigenic proteins represent proteins for gene expression (13%) and chaperons (10%). Until now 60 B cell epitope peptides (24-30 aa) have been predicted from 35 proteins. Additionally we use sera from naturally infected animals in order to identify immunogenic proteins from which further epitope peptides can be derived, as most published studies used human sera or sera from experimentally infected rodents. Thus our proteomic approach circumvents the problem of antigenic difference between host species and completes antigen identification. For this purpose 2D gel electrophoresis of *C. burnetii* proteins, western blotting and spot matching have now been established in our laboratory for identification via mass spectrometry.
Initial results from literature review and *in silico* analyses yielded in first 60 predicted B cell epitopes. Approximately a total of 150 peptides will be predicted from both the literature and proteomic approach. These will be selected with field sera for strong and specific recognition. Finally a combination of peptides will be used for the development of a routine Q fever diagnostic method.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

630/KMP
Prevalence of streptococcal virulence factors in *Streptococcus dysgalactiae* subsp. *equisimilis* clinical strains isolated from humans and animals.
M. Ciszewski*[1], E. Szewczyk[1]
[1]*Medical University of Lodz, Department of Pharmaceutical Microbiology and Microbiological Diagnostics, Lodz, Poland*

Introduction: *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) is a pyogenic species pathogenic both for humans and animals. Until recently, it has been considered an exclusive animal pathogen causing infections in wild as well as domestic animals. Currently, human infections are being reported with increasing frequency. Their clinical picture is often similar to the ones caused by *Streptococcus pyogenes*. This species seems to be a proper example of changes which continually occur in microorganisms and their implications for human health.
Objectives: The aim of this study was to determine phenotypic and genotypic factors conditioning adhesion, evasion of host immune system, synthesis of invasins, toxins and superantigens in SDSE isolates derived both from humans and animals. Detection of these factors in respective strains might indicate the direction of a gene transfer and identify the factor that might enable animal-to-human transfer.
Materials & methods: The subject of this study consisted of 30 SDSE strains isolated from clinical samples from humans in hospitals in Łódź, Poland. Additionaly, a group of 6 SDSE strains obtained from veterinary laboratory in the same area, isolated from clinical cases from dogs has also been analyzed.
The isolates ability to produce some of the virulence factors, such as proteases, lipases, streptokinase able to activate human plasminogen, BLIS substances inhibiting growth of human skin microbiota, were determined phenotypically. Strains hemolytic properties were also examined. Moreover *emm* typing and the presence of 26 virulence genes in genomic DNA was evaluated.
Results: The prevalence of virulence factors in two examined groups of strains was substantially different. Some virulence factors, such as M protein, streptolysin O and fibronectin-binding protein were present only in human SDSE isolates. Human SDSE isolates showed also greater colonization potential, as they produced BLIS substances inhibiting growth of *Corynebacterium* spp. – a substantial element of human skin microbiota. The overall number of present virulence factors were also essentially lower in SDSE animal isolates.
Conclusion: Obtained results suggests that SDSE strains might asymptomatically transfer to human skin, enriching its microbiota. At the time they might acquire virulence genes from commensal human streptococci or *S. pyogenes*. As a result SDSE might become an opportunistic pathogen and develop full infection in favorable conditions, i.e. skin damage or immunological system impairment. Virulence factors identified only in human SDSE isolates should be regarded as the factors enabling strains to develop human infections.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

631/KMP
Detection of methicillin-resistant coagulase negative staphylococci by MALDI-TOF MS
D. Schuster*[1], M. Josten[1], I. Bodenstein[1], M. Gajdiss[1], C. Szekat[1], H. G. Sahl[1], G. Bierbaum[1]
[1]*University Clinics of Bonn, Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), Bonn, Germany*

Introduction: An ever increasing number of methicillin-resistant coagulase-negative staphylococci (MR-CONS) is isolated in hospitals. These strains cause nosocomial infections and represent - since they are often resistant to penicillinase-stable β-lactams - a substantial health care burden. The most frequently encountered CONS in hospital is *S. epidermidis*, but other species like the more pathogenic *S. lugdunensis* are common as well.
Objectives: The aim of this study was to test whether identification of methicillin resistance in staphylococci from patient samples by routine matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) is possible.
Material & methods: In previous work we have shown that agr-positive nosocomial methicillin-resistant Staphylococcus aureus (MRSA), which possess SCC-mec cassettes of type II, III or VIII, excrete a small peptide. This peptide is called PSM-mec and encoded on these three SCC-mec cassettes. The peptide PSM-mec is visible at *m/z* 2415 during MALDI-TOF MS of whole cells and using this method it is possible to identify MRSA by analyzing the spectra during mass spectrometry. In view of the fact that MR-CONS and MRSA acquired similar SCC-mec cassettes we established a collection of clinical CONS and evaluated the collection by PCR for the presence of the structural gene encoding PSM-mec and the appearance of the corresponding signal during mass spectroscopy.
Results: So far, more than 350 strains have been characterized, among them 73 % *S. epidermidis*, but also *S. haemolyticus* (10 %), *S. hominis* (9 %), *S. capitis*, *S. lugdunensis*, *S. simulans*, *S. warneri*, *S. schleiferi*, *S. caprae*, *S. cohnii*, and S. auricularis. 76 % of all isolates were methicillin-resistant, however, only 27 % of all isolates (or 35 % of all resistant isolates) contained the gene *psm-mec*. In MALDI-TOF MS spectra, 83 % of the strains that harbored the gene yielded the correct signal. There were no false positive results.
Conclusion: In conclusion, a signal at *m/z* 2415 in a spectrum of CONS indicates methicillin resistance.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

632/KMP
Identification of a peptidoglycan hydrolase in chlamydiae
J. Reuter*[1], H. Bühl[1], M. Brunke[1], A. Klöckner[1], B. Henrichfreise[1]
[1]*University of Bonn, Institute for Pharmaceutical Microbiology, Bonn, Germany*

The obligate intracellular *Chlamydiaceae* do not face osmotic challenges and thus lack a stabilizing peptidoglycan (PG) envelope. Nevertheless, they build a transient PG ring at the septum of dividing cells. Since the septum separates the two dividing daughter cells, the PG ring must be remodeled during constriction and degraded afterwards. For this process, PG hydrolysing enzymes are required. So far, neither lytic transglycosylases nor endopeptidases, which are major PG-remodeling enzymes in cell-wall wrapped bacteria, have been found in *Chlamydiaceae*. Here, we identified a PG hydrolase in *Chlamydia pneumoniae* and started to investigate its biological function. The chlamydial protein was analyzed using (i) *in vitro* activity assays with the purified enzyme and either the monomeric PG precursor lipid II or remazol-stained *E. coli* PG sacculi as a substrate, (ii) active site mutagenesis studies and (iii) *in vivo* lysis assays in *E. coli*. The protein was capable of hydrolysing purified PG but did not use lipid II as a substrate. Moreover, the enzyme had a lytic effect on *E. coli* and its activity was dependent on a functional Cys-His-His triad in the active site. The identification of a PG hydrolase helps to elucidate PG ring remodelling in chlamydiae. Further research is needed to understand how a minimal and modified PG biosynthesis machinery supports cell division in chlamydiae.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

633/KMP
Influence of respiratory enzymes on virulence of *Vibrio cholerae*
B. Forler*[1], F. Zingl[2], S. Schild[2], J. Steuber[1]
[1]*University of Hohenheim, Department of Microbiology, Stuttgart, Germany*
[2]*University of Graz, Institute for Mol. Biosciences , Graz, Austria*

Question: The periplasmic nitrate reductase plays an important role in pathogenesis for *Salmonella enterica* serovar Typhimurium. Using a mouse model it was shown that *napA* deletion strains, which lack the catalytic subunit of the periplasmic nitrate reductase, show a distinct growth defect in the lumen of the colon (1). Although *Vibrio cholerae* , unlike *S.* Typhimurium, lacks the successive nitrite reductase (2), *napA* is strongly expressed during infection (3). In order to investigate the role of the periplasmic nitrate reductase during infection for *V. cholerae*, *napA* deletion strains were constructed. Both *in vitro* and *in vivo* experiments are required to point out the significance of the periplasmic nitrate reductase in the infection process of a host.
Methods: *NapA* deletion strains were constructed with a plasmid based on the suicide plasmid pCVD442, that contains an up- and downstream fragment of *napA* and a resistance cassette. Growth experiments under anaerobic conditions with two *V. cholerae* strains were conducted in M63 minimal medium with 0.2 % glycerol as electron donor and different terminal electron acceptors (fumarate, TMAO, DMSO, or nitrate). As a control, cells were also grown in M63 medium with glucose or glycerol only. Nitrate was added as potassium salt (5 mM). Growth was followed by measuring the optical densities of cell cultures at 600 nm. Nitrite concentrations of the supernatants of the cell cultures were determined spectrophotometrically from the formation of a chromophore based on the Griess reaction.
Results: Positive clones with a deletion of the whole *napA* were obtained. Anaerobic growth was observed with fumarate or TMAO, but not with nitrate, DMSO, or without electron acceptor. Importantly, growth yields observed with glucose only were higher than yields observed with glucose plus nitrate. Up to 10 µM nitrite was measured in samples with glycerol plus nitrate and more than 100 µM in samples with glucose and nitrate. No nitrite was measured in samples with glucose and glycerol plus disodium fumarate, TMAO, DMSO.
Conclusion: *V. cholerae* lacks a nitrite reductase which detoxifies nitrite (2), which explains why nitrite accumulated with cells grown in the presence of nitrate. Growth experiments, nitrite assays and animal studies with *napA* deletion strains of *V. cholerae* for further characterization of these strains are ongoing.

References
[1] Lopez C a., Rivera-Chávez F, Byndloss MX, Bäumler A. The periplasmic nitrate reductase *NapA*BC supports luminal growth of *Salmonella enterica* serovar Typhimurium during colitis. *Infect Immun*. 2015 2015; 83(9):3470-8
[2] Braun M, Thöny-Meyer L. Cytochrome c maturation and the physiological role of c-type cytochromes in *Vibrio cholerae*. *J Bacteriol*. 2005;187(17):5996-6004

[3] Nielsen AT, Dolganov NA, Otto G, Miller MC, Cheng YW, Schoolnik GK. RpoS controls the *Vibrio cholerae* mucosal escape response. *PLoS Pathog*. 2006;2(10):0933-0948.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

634/KMP

Investigating the role of Cyclophilins in the virulence of *Burkholderia pseudomallei*

M. Sarkar-Tyson*[1], N. Bzdyl[1], T. Inglis[1], C. Kahler[1]
[1]*University of Western Australia, Perth, Australia*

Question: Cyclophilins are a family of proteins which exhibit peptidyl-prolyl *cis-trans* isomerase (PPIase) activity which results in the *cis* to *trans* isoform change of xaa-proline bonds, a rate limiting step of protein folding. Cyclophilins have been identified in nearly all living organisms and are associated with virulence in intracellular bacteria. Due to the highly conserved nature, cyclophilins are potential novel anti-virulence targets for many pathogenic bacteria, such as *Burkholderia pseudomallei*, which encodes two cyclophilin genes, *ppiA* and *ppiB. B. pseudomallei* is the causative agent of melioidosis, which is endemic in South-East Asia and Northern Australia. Due to the high levels of antibiotic resistance and lack of effective treatment for Melioidosis, new medical countermeasures are urgently needed. This study investigates the potential for cyclophilins as novel anti-virulence targets. To understand the role of cyclophilins in *B. pseudomallei* virulence, a Cyclophilin A knockout mutant was constructed and characterised.
Methods: A *B.pseudomalleiΔppiA* mutant strain was created and confirmed by PCR and sequencing. The ability of *B.pseudomalleiΔppiA* to invade and survive in macrophage cells was investigated. The mutant strain was tested for susceptibility to intracellular stresses. The ability of *B.pseudomalleiΔppiA to form* Biofilms was also investigated.
Results: *B.pseudomalleiΔppiA* showed decreased levels of intracellular bacteria up to 6 hours post infection and demonstrated increased sensitivity to oxidative stress. *B.pseudomalleiΔppiA* had decreased biofilm formation showing a 7-fold decrease in absorbance following biofilm-stimulating conditions after 48 hours.
Conclusions: Deletion of *B. pseudomallei ppiA* results in decreased virulence and biofilm formation. This demonstrates that cyclophilins play an important role in the virulence of *B. pseudomallei.* Cyclophilin A presents as a novel anti-virulence target in this bacterium.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

635/KMP

Computational analyses of different mycobacterium species to identify unique proteins of *Mycobacterium tuberculosis*

S. K. Arora*[1], J. Ahmad[1], M. P[1], R. Sharma[1], S. A. Rahman[2], S. E. Hasnain[3,4,5], N. Z. Ehtesham[1]
[1]*National Institute of Pathology, Inflammation Biology and Cell Signaling Laboratory, New Delhi, India*
[2]*European Molecular Biology Laboratory, Welcome Trust Genome Campus, European Bioinformatics Institute, Hinxton, United Kingdom*
[3]*Dr. Reddy's Institute of Life Sciences, University of Hyderabad Campus, Professor CR Rao Road, Hyderabad, India*
[4]*Molecular Infection and Functional Biology Laboratory, Kusuma School of Biological Sciences, Indian Institute of Technology, New Delhi, India*
[5]*JH-Institute of Molecular Medicine, New Delhi, India*

Tuberculosis, caused by *Mycobacterium tuberculosis (M.tb)*, a member of the genus Mycobacterium remains a major global health problem and is one of the world's deadliest communicable diseases. Genus Mycobacterium comprises more than 120 species with only few being pathogenic to humans. In a few cases, *Mycobacterium bovis* has also been reported to be the causative organism of this disease. We performed comparative proteomic analysis of 13 mycobacterium species from different categories of genus mycobacteria to identify the unique proteins. These species included the: Strict pathogens (most virulent pathogens), Opportunistic pathogens, which belong to Non-Tuberculous Mycobacteria (NTM) group, can cause pulmonary and other disseminated infections in immune compromised individuals and the Non-pathogenic group which do not cause disseminated infections even in immune compromised individuals. Our study revealed 25 proteins unique to *Mycobacterium tuberculosis* that shared less than 20% sequence identity with other proteins of Mycobacterium species used in the analysis. We further categorized these 25 proteins in 6 categories based on database information and literature review. We exploited their diagnostic potential both at the nucleotide and protein level by doing BLASTn of identified unique proteins against all proteins in the database. This led to the identification of 6 proteins as *M.tb* signature proteins. After further analysis, 4 DNA signature probes for PCR-based diagnostic assay for tuberculosis were shortlisted and validated using *M.tb* genomic DNA isolated from tuberculosis patients.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

636/KMP

Molecular and functional characterization of the ser/thr protein kinase PknB and phosphatase Stp of *Staphylococcus aureus*.

M. Jarick*[1], M. Steger[2], A. Schlosser[2], K. Ohlsen[1]
[1]*Universität Würzburg, Institut für Molekulare Infektionsbiologie , Würzburg, Germany*
[2]*Universität Würzburg, Rudolf-Virchow-Zentrum für Experimentelle Biomedizin, Würzburg, Germany*

Staphylococcus aureus is one of the most common causes of community and nosocomial infections giving rise to life-threatening conditions including bacteremia, endocarditis, sepsis, or toxic shock syndrome. The activity of many metabolic proteins and virulence factors is controlled by phosphorylation and dephosphorylation via kinases and phosphatases.
PknB (Stk1) is a eukaryote-like serine/threonine kinase, which acts in signal transduction of *S. aureus* through reversible phosphorylation of target proteins. PknB and its cognate phosphatase Stp are involved in central metabolic processes like cell wall metabolism, purine synthesis, glycolysis and importantly affect also *S. aureus* virulence.
At present, several putative phosphorylation targets of PknB are identified. We aim to decipher the role of PknB and Stp on central metabolism as well as its interplay with classical two-component systems.
Western Blot analysis revealed a strong impact of PknB and Stp on the phosphoproteome of *S. aureus* suggesting that both proteins are global protein regulators with overlapping target protein specificity. In this study, we used the bacterial two-hybrid system to validate possible interaction partners of PknB and Stp. PknB and Stp seem to interact with cell wall synthesis and cell division proteins. Eventually, we intend to reconstruct the regulatory network surrounding the counteraction of PknB with the phosphatase Stp.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

637/KMP
Shaping the human nasal microbiota by the antibiotic lugdunin
C. Laux*[1], B. Krismer[1], A. Peschel[1]
[1]*University of Tübingen, Infection Biology, Tübingen, Germany*

A nasal *Staphylococcus lugdunensis* strain, producing a remarkable antibacterial activity against *Staphylococcus aureus* and other major human pathogens, was identified and characterized. The antimicrobial compound was found to be a non-ribosomally synthesized peptide antibiotic named lugdunin. Lugdunin is a novel, cyclic thiazolidine-containing compound, which enables *S. lugdunensis* to eradicate *S. aureus* in vitro and in animal models. The human nasal colonization by *S. lugdunensis* was associated with a significantly reduced *S. aureus* carriage rate. This was shown in two independent studies of high risk patients as well as healthy people. Until now the regulation of lugdunin is still unknown. For this purpose, an investigation of the regulatory circuits has been started via a reporter system assay - preliminary results are already obtained.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

638/KMP
CPP-peptidoglycosidase fusion proteins targeting intracellular bacterial pathogens
T. Fernandes Cunha Martins*[1], I. Benz[1], C. Rüter[1], A. Schmidt[1]
[1]*Centre for Molecular Biology of Inflammation - ZMBE, Institute of Infectiology, Münster, Germany*

A novel cell-penetrating peptide (CPP), Yersinia effector protein YopM, was identified with the ability to cross cellular membranes either alone or in association with bioactive cargo. This activity of YopM might serve as self-delivering immune modulatory protein for therapeutic application. Endolysins have attracted great interest as novel antimicrobials against Gram-positive bacteria. Fusions of CPP with endolysins may represent a novel and innovative option for the generation of antimicrobials against intracellular bacterial pathogens.
For this purpose fusion proteins of recombinant variants of endolysins with CPP have been generated, expressed, isolated and purified. These fusion proteins will be assessed with respect to their cell-penetrating ability in various cell lines including immune cells. Furthermore, we have characterized their putative antibacterial activity against various Gram-positive and also Gram-negative pathogens by using confocal microscopy, flow cytometry, immunoblotting assay and by performing a Zymogram analysis to determine peptidoglycan (PG) degradation activity.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

639/KMP
Restricted usefulness of novel β-lactam/β-lactamase inhibitor combinations – results from a one year retrospective study on multidrug-resistant Gram-negative organisms in a German University Medical Center
J. Katchanov[1,2], E. M. Klupp[3], L. Asar[3,1], H. Rohde[3], S. Kluge[2], F. Maurer*[3,1]
[1]*University Medical Center Hamburg-Eppendorf, Antimicrobial Stewardship Team, Hamburg, Germany*
[2]*University Medical Center Hamburg-Eppendorf, Department of Intensive Care Medicine, Hamburg, Germany*
[3]*University Medical Center Hamburg-Eppendorf, Institute of Medical Microbiology, Virology and Hygiene, Hamburg, Germany*

Background: The purpose of this study was to determine the burden and spectrum of infections with multidrug-resistant Gram-negative pathogens (4MRGN) in hospitalised patients in a German academic tertiary care center. We aimed to characterise i) mechanisms of carbapenem resistance, ii) drug susceptibility towards last resort antimicrobials, iii) modes of 4MRGN acquisition, and iv) clinical outcomes of the affected patients.
Methods: We analyzed electronic medical records for all patients, in whom 4MRGN (Gram-negative bacteria showing resistance to carbapenems, acylureidopenicillins, third generation cephalosporins and fluoroquinolones) were detected from September 1, 2015 until August 31, 2016. In addition, microbiology data of all 4MRGN isolates was reviewed with respect to their drug-susceptibility profile and carbapenemase genotype.
Results: 117 individual patients were colonized or infected with 4MRGN pathogens. The species distribution was *Pseudomonas aeruginosa,* n=64; *Acinetobacter baumanii,* n=18; and *Enterobacteriaceae spp*., n=44. Antimicrobial treatment with broad spectrum antibiotics was documented in 103 patients (88.0%). 73 patients (62.4%) received a carbapenem, 5 patients received ceftazidim/avibactam (in-hospital mortality 5/5, 100%) and 5 received ceftolozan/tazobactam (in-hospital mortality 3/5, 60%). Evidence for nosocomial transmission was obtained in 29 patients (24.8%). In 25 patients (21.4%), a 3MRGN (carbapenem-susceptible) isolate was detected 36 days (IQR 22,25-74,25) prior to detection of a 4MRGN isolate. 12 patients (9.4%) had documented previous contact to the health system in a country with high 4MRGN prevalence. Genes encoding carbapenemases were detected in 60 individual patient isolates (51.3%). The distribution was class B (NDM-1, VIM-2), n=29; and Class D (OXA-23, OXA-48), n=31. During the study period, no class A carbapenemase and no co-expression of more than one carbapenemase was detected.
Conclusion: Our data suggest a high risk for development of carbapenem-resistance due to selective pressure under prolonged antimicrobial therapy, which is a more common source for 4MRGN colonization/infection than previous foreign travel. In addition, nosocomial transmission is a major source of 4MRGN acquisition. First experiences with novel β-lactam/β-lactamase inhibitor combinations show that their benefit is limited in our setting, likely because almost half of the carbapenemase-producing strains included in this study expressed a class B enzyme.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

640/KMP
The impact of multidrug resistance on the fitness of *Pseudomonas aeruginosa* under different environmental conditions
A. Seupt*[1], S. Bruchmann[1], M. Schniederjans[1], S. Häußler[1]
[1]*Helmholtz Centre for Infection Research, Molecular Bacteriology, Braunschweig, Germany*

Question: This study sought to investigate the fitness effect of mutations leading to antibiotic resistance (gyrase mutations conferring resistance to fluoroquinolones) and multidrug resistance (overexpression of RND efflux pumps) in the Gram-negative opportunistic human pathogen *Pseudomonas aeruginosa* under different environmental conditions. *P. aeruginosa* is known to be a highly adaptive species that is able to develop resistances to a variety of antibiotic compounds of different classes[1,2].
Materials/Methods: Fitness effects were assayed *in vitro* under rich media conditions, under osmotic and oxidative stress in competition experiments. The strains of interest were incubated in 1:1 ratio with a reference strain over a period of 96 hours.
Furthermore, the relative fitness of one efflux pump knockout mutant was assayed *in vivo* using larvae of *Galleria mellonella* as an infection model. The larvae were inoculated with a 1:1 mixture of the reference strain and the strain of interest. The larvae were

incubated for approximately 24 hours, larval hemolymph was isolated and used for a second infection of larvae.
The resulting ratio of reference (wild type) to mutant strain was determined via pyrosequencing-based SNP allele frequency estimation.
Results: Overexpression of the two most important RND efflux systems regarding multidrug resistance (MexAB-OprM and MexXY) did only result in minor (MexAB-OprM under osmotic stress) to no fitness disadvantage compared to the wild type *in vitro*.
Interestingly, the deletion of *mexXY* was shown to cause a fitness advantage of the analyzed strain over the wild type *in vivo*.
The overexpression of the two efflux systems MexCD-OprJ and MexEF-OprN did result in significant fitness disadvantages *in vitro* except for the overexpression of MexCD-OprJ under oxidative stress conditions.
The analyzed gyrase mutants did not show any significant fitness disadvantage over the wild type in any of the tested conditions *in vitro*.
Conclusion: It could be shown that antibiotic resistance conferring mutations in efflux pump regulators and gyrase genes is not necessarily correlated to a significant fitness burden. Moreover, the findings of this study further underline the adaptability of *P. aeruginosa* to different environments. Additionally, the usage of pyrosequencing as a technology to analyze such fitness assays was shown to be highly applicable for answering the asked questions. Furthermore, the study underlined the importance of *in vivo* assays to assess the impact of resistance conferring mutations on the fitness of *P. aeruginosa*.

References
[1] Khaledi, A. *et al.* Transcriptome profiling of antimicrobial resistance in Pseudomonas aeruginosa. *Antimicrob. Agents Chemother.* (2016). doi:10.1128/AAC.00075-16
[2] Breidenstein, E. B. M., de la Fuente-Núñez, C. & Hancock, R. E. W. Pseudomonas aeruginosa: All roads lead to resistance. *Trends Microbiol.* 19, 419–426 (2011).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

641/KMP
Identifying genetic determinants of antibiotic resistance in clinical isolates of *P. aeruginosa* by Transposon-Sequencing
S. Schinner*[1], M. Schniederjans[1], A. Khaledi[1], S. Bruchmann[1], S. Häußler[1]
[1]*Helmholtz Centre for Infection Research, Molecular Bacteriology, Braunschweig, Germany*

Question: *Pseudomonas aeruginosa*"s ability to acquire new antimicrobial resistances during infection makes it a very challenging bacterium for current diagnostics and therapies. Thus, there is a strong need for determining key genetic determinants of antibiotic resistances. In a previous study, the genetic modifications underlying antibiotic resistance phenotypes were investigated in a large collection of clinical isolates 1. Even though the majority of antibiotic resistance traits could be identified, some resistance phenotypes could not be correlated to known genetic markers. In order to identify putative novel antibiotic resistance determinants, we apply high-throughput sequencing technology transposon sequencing (Tn-Seq).
Methods: Three clinical isolates whose ciprofloxacin (MIC= 4 µg/ml) or tobramycin (MIC= 16 and 512 µg/ml) resistance phenotypes could not be explained on the basis of sequence variations and gene expression by RNA-Seq alone were further analyzed1. As no previously described resistance determinants were identified, Tn-Seq is performed on the selected clinical isolates. After transposon insertional mutagenesis, changes in the make-up of transposon mutant pools grown under selection by tobramycin or ciprofloxacin sub-MIC level conditions is monitored by high-throughput sequencing. After library selection, frequency changes of each insertion mutant are determined by sequencing the flanking regions. These changes are used to calculate each mutant's fitness.
Results: RNA sequencing identified key genetic determinants of antibiotic resistance for most of the isolates1. Ciprofloxacin resistance of the selected clinical isolate could not be explained by target mutations in quinolone resistance-determining regions. Tobramycin resistance of the remaining two isolates could not be referred to aminoglycoside modifying enzymes.
The generation of transposon mutant libraries was established in *P. aeruginosa* clinical isolates for Tn-seq. Mapped reads resulting from sequencing of the transposons" flanking regions determine the frequency of each insertion mutant. Transposon mutants which are depleted after sub-MIC selection, lack an essential gene conferring antibiotic resistance.
Conclusions: Using RNA-Seq as well as other screening methods many but not all genetic determinants underlying antibiotic resistance can be identified1. Tn-Seq is a novel methodology that is used here for the identification of previously uncharacterized resistance related genetic regions that might remain undetected in traditional procedures.

References
[1] Khaledi A, Schniederjans M, Pohl S, Rainer R, Bodenhofer U, Xia B, Klawonn F, Bruchmann S, Preusse M, Eckweiler D, Dötsch A, Häussler S. 2016. Transcriptome profiling of antimicrobial resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 60:4722–4733. doi:1128/AAC.00075-16.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

642/KMP
Teixobactin Induced Lysis in *Staphylococcus aureus*
J. Deisinger*[1], I. Engels[1], A. Müller[1], F. Götz[2], B. Conlon[3], K. Lewis[3], T. Schneider[1]
[1]*IFMB Bonn, Pharmaceutical Microbiology, Bonn, Germany*
[2]*University of Tübingen, Microbial Genetics, Tübingen, Germany*
[3]*Northeastern University, Biology, Boston, Massachusetts, United States*

The rapid emergence of infections caused by multidrug-resistant pathogens in community and hospital settings represents a serious public health problem. Novel antibiotics with unprecedented modes of action are urgently needed to overcome rising resistance. Analysis of the mechanism of action of an antibiotic and identification of the target structure are integral components of the drug development process.
Teixobactin, recently isolated by screening previously uncultured bacteria, is highly potent against gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *S. aureus* (VRSA), vancomycin-resistant *Enterococci* (VRE) and *Mycobacterium tuberculosis*. A previous study showed that teixobactin selectively inhibits cell wall biosynthesis reactions by binding to peptidoglycan precursor lipid II and wall teichoic acid precursor lipid III with high affinity (Ling and Schneider *et al*., 2015). Furthermore, teixobactin shows potent bactericidal activity indicating that another yet uncharacterized mechanism may be triggered by this compound.
The impact of teixobactin on membrane integrity, wall teichoic acid biosynthesis, as well as on the autolytic system was analyzed to gain further information on the cellular effects underlying lysis.

References
[1] Ling, L. L., Schneider, T., Peoples, A. J., Spoering, A. L., Engels, I., Conlon, B. P., Müller, A., Schäberle, T. F., Hughes, D. E., Epstein, S., Jones, M., Lazarides, L., Steadman, V. A., Cohen, D. R., Felix, C. R., Fetterman, K. A., Millett, W. P., Nitti, A. G., Zullo, A. M., Chen, C., and Lewis, K. (2015). A new antibiotic

kills pathogens without detectable resistance. Nature, 517(7535):455_459.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

643/KMP
Analysis of SEDS protein family members from *Staphylococcus aureus*
J. Zimmermann*[1], P. Hardt[1], T. Schneider[1], F. Grein[1]
[1]*University Bonn, Pharmaceutical Microbiology, Bonn, Germany*

The healthcare-associated pathogen *Staphylococcus aureus* causes a variety of infections, most notably soft tissue, skin, bone and bloodstream infections and is also the most common cause of postoperative wound infections.
The use of ß-lactams antibiotics, which have long been the first choice for the treatment of staphylococcal infections, is currently significantly impaired by the increasing spread of non-susceptible strains leading to an urgent need for novel antiinfectives. Penicillin binding proteins (PBPs), which represent the main target for ß-lactam antibiotics, are long known to be responsible for the polymerization of the peptidoglycan network. However, recently the broadly conserved proteins out of the shape, elongation, division, sporulation (SEDS-) family were identified as novel class of peptidoglycan glycosyltransferases in rod-shaped bacteria by Meeske et al (2016)[1]. Here we report on the heterologous expression, purification and functional analysis of SEDS proteins from Staphylococcus aureus and the *in vivo* localization of fluorescently labeled derivatives of these proteins.

References
[1] Meeske, A. J. et al. SEDS proteins are a widespread family of bacterial cell wall polymerases. Nature 537, 634–638 (2016)

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

644/KMP
Ribosome profiling in pseudomonas aeruginosa
L. Pezoldt*[1], A. Khaledi[1], S. Häußler[1]
[1]*Helmholtz centre for infection research, Braunschweig, Germany*

Question: Aim of the project is to unravel the variability of pseudomonas aeruginosa on a translational level and conclude to the overall protein expression of the species under certain conditions.
Methods: To that end Ribosomal Footprint Profiles of an assortment of our pseudomonas clinical isolates collection will be generated in parallel to RNAseq analysis.
Results: The results will be compared with our broad investigation of the clinical isolates phenotypes (Antibiotic resistance, Biofilm formation, Virulence).
Bioinformatic analyses will cluster the strains in phenotypic groups with shared patterns in their ribosome profile. The focus will then be set on subgroups of strains that show clinical relevant phenotypes.
Conclusion: We aim to unravel regulatory mechanisms that lead to a differential expression of the same mRNA pool which then manifests a different phenotype.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

645/KMP
Risk factors for death in septic trauma patients
R. Otchwemah*[1], R. Lefering[2], T. Tjardes[3], B. Bouillon[3], F. Mattner[1]
[1]*Kliniken Köln, Institute for Hygiene, Cologne, Germany*
[2]*Universität Witten/Herdecke in Köln-Merheim, IFOM, Institut für Forschung in der operativen Medizin, Cologne, Germany*
[3]*Cologne-Merheim Medical Center, Department of Traumatology and Orthopedic Surgery, Cologne, Germany*

Introduction: Severely injured patients are at risk of sepsis. Physiologic adaptions to trauma, the need for immobilisation, many hand-ons and devices may be reasons for that. While over all trauma mortality has been reduced over the last decades, mortality in the subgroup of trauma patients, who have developed a sepsis, remained at the same level.
Objectives: While risk factors for death in all trauma patients include injury severity, as the most important prognostic factor, massive bleeding, multiple organ failure (MOF) and age, little is known about those risk factors in the sepsis subgroup. The presented study tries to identify risk factors for death in the subgroup of trauma patients with sepsis.
Patients & methods: In a retrospective cohort study we analysed electronic medical records of all trauma patients with an injury severity score (ISS) > 8, who were admitted to intensive care unit of our level 1 trauma centre from 2012 to 2014 and developed a sepsis during their stay. Patients were identified by using the TraumaRegister DGU® of the German Trauma Society. Sepsis was defined as systemic inflammatory response to an infection following the ACCP/SCCM Consensus Conference recommendations. Only the first septic event was considered. We identified gender, age, ISS, number of red blood cell units transfused, MOF, responsible infection, time until onset of sepsis, detected pathogens, multiple drug resistance and the used calculated antibiotic therapy. For mathematical analysis four fields blackboard, chi-squared test and standard deviations were used.
Results: We included 101 Patients at a mean age of 53.2 (± 20.2) years. Seventy- seven (76%) were male, 24 (24%) female. Eighty patients (79%) survived, 21 (21%) died. Mean ISS was 30 (±14). Mean number of red blood cell units transfused was 1.5 (± 3.3). In 91 (90%) patients a MOF was documented. Mean onset of sepsis was 4 (±3.1) days after trauma. One-hundred seven clinical infections with systemic responses were identified: 86 cases of pneumonia (80%), 8 urinary tract infections (7%), 8 times infection was not specified (7%), 3 primary bloodstream infections (3%), 1 peritonitis (1%) and 1 skin abscess (1%). Gram-positive strains were found in 42 (37%) and gram-negative in 72 (64%) cases. Seven (7%) Patients developed infections with multiple drug resistant bacteria (6 gram-negative, 1 MRSA). Comparing survivors with non-survivors, age was the only factor, which demonstrated a significant difference (survivors: 49.6 (±19.1) years; non-survivors: 66.7 (±18.7) years; p= 0.001). The other parameters, especially ISS, did not differ significantly.
Conclusion: Among the group of trauma patients with sepsis a higher age, not severity of injury seems to be the most important risk factor for death. This result emphasises the imperative necessity of strict infection control measures in the elderly, regardless of injury severity.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

646/KMP

Treatment with probiotics ameliorates stress-induced pathological aggression in mice

D. Pavlov*[1], A. Gorlova[1], E. Zubkov[2], T. Cherdyntseva[1], O. Karpuchina[1], A. Inozemtsev[1], T. Strekalova[3], V. Chekhonin [2]
[1]*Lomonosov Moscow State University, Neuroscience, Moscow, Russian Federation*
[2]*Serbsky Institute of psychiatry and narcology, Immunochemistry, Moscow, Russian Federation*
[3]*Maastricht University, School for Mental Health and Neuroscience, Maastricht, Netherlands*

Pathological aggression is one of the increasing problems in human society nowadays. Limited number of remedies are available to manage high aggressiveness in a clinic. In current study, we used chronic exposure of male Balb/c mice to an ultrasound (US) of randomly changing negative/ neutral frequencies (22-45 kHz), for 21 days, to induce a state of high aggressiveness. Recent studies showed that lacto- and bifidobacteria can affect brain function in various ways generating beneficial behavioural effects on social behavior in mammals. Based on that, here, we investigated the effects of a probiotic culture with Bifidobacterium spp. and Lactobacillus spp. (in equimolar concentrations) at the dose of 1×10^9 CFU mL^{-1} on aggressive behavior in the above-mentioned model. Atypical antidepressant buspirone which was applied at the dose 30 mg/kg, served as a reference treatment. Our data showed that mice that were chronically exposed to the US displayed pathological aggression and dramatic impairment of memory, as well as increased anxiety-like behaviors. We also found a reduction of 5-HTR1a and significant increase of BDNF and 5-HT2a expression in the cerebellum, prefrontal cortex, amygdala and hippocampus of US-mice in comparison with vehicle-treated animals. The administration of buspirone or a combination of probiotics ameliorated pathological aggressiveness of chronically stressed mice. These effects had different times of onset in a course of a treatment: on day 14 for a treatment with buspirone and on day 29 for a treatment with probiotics. The improvement of social behavior in the US-challenged mice was accompanied by ameliorated scores of cognitive abilities and anxiety. Buspirone-treated stressed mice showed no differences in 5-HT1a and 5-HT2a mRNA levels, as compared with stressed non-treated animals. However, probiotic-fed US-challenged animals showed unaltered mRNAs levels of these genes as compared with control group. While the mechanisms of ameliorative effects of probiotics remain to be investigated, it is remarkable that employed here interventions can effectively counteract cognitive and emotional disturbances that result from chronic emotional stress. Thus, a modification of a diet could be an effective way of amelioration severe cognitive disturbances that worth to be investigated further.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

647/KMP

Identification and characterization of genetic determinants of the small colony variant phenotype in *Pseudomonas aeruginosa*

M. Koska*[1], M. Schniederjans[2], A. Kordes[1], A. Khaledi[2], V. Kaever[3], S. Häußler[2,1]
[1]*TWINCORE GmbH, Centre of Clinical and Experimental Infection Research, a joint venture of the Hannover Medical School and the Helmholtz Center for Infection Research, Institute for Molecular Bacteriology, Hannover, Germany*
[2]*Helmholtz Center for Infection Research, Department of Molecular Bacteriology, Braunschweig, Germany*
[3]*Hannover Medical School, Research Core Unit Metabolomics, Hannover, Germany*

Introduction: The intracellular second messenger 3´,5´-cyclic-di-guanosine monophosphate (c-di-GMP) modulates diverse functions as rhamnolipid biosynthesis, exopolysaccharide production, siderophore production, virulence, cell division and motility in many bacteria [1]. Besides that, c-di-GMP not only directs surface attachment and motility behavior, but was also shown to be triggered upon surface contact [2]. Thus, c-di-GMP most likely possess a key role in lifestyle switch from the virulent state in acute infections to the less virulent but more resilient biofilm state characteristic for chronic infectious diseases.
Various studies demonstrated a strong link between elevated levels of c-di-GMP and a small colony variant (SCV) phenotype in *P. aeruginosa* [3]. These highly adapted subpopulations are frequently isolated from the chronically infected CF lung and correlate with increased antibiotic resistance, poor lung function [4]. However, genetic determinants leading to the SCV phenotype are still not well understood. Modulating c-di-GMP signaling pathways in bacteria could represent a new way of controlling formation and dispersal of biofilms in medical and industrial settings.
Objectives: The aim of this work is the correlation of phenotype and genotype of SCVs and respective revertants to identify and characterize new determinants which could explain high levels of c-di-GMP in the SCV phenotype in *P. aeruginosa*.
Materials & methods: Having access to a strain collection of 150 clinical CF isolates harboring an SCV phenotype, we passaged chosen SCVs till they gave rise to nonaggregative revertants that produce large colonies on agar plates. Both, SCV and revertant, were than compared with regard to colony morphology, motility behavior, biofilm formation, antibiotic resistance, virulence and c-di-GMP concentration. The most promising SCV-revertant pairs were subjected to whole-genome and transcriptome sequencing.
Results: Despite huge differences in the colony morphologies between the SCVs and the respective revertants, often no clear pattern were observed in the other phenotypes except motility. Using whole genome sequencing we could identify various single nucleotide polymorphisms (SNPs) comparing SCVs with the corresponding revertants. In some clinical isolates we found interesting candidate genes which might be responsible for the SCV phenotype. In others, there was not a single overlap in affected genes comparing the revertants among each other.
Conclusion: The SCV adaption cannot be explained by a unique gene locus or certain pathway but seems to be influenced by various mechanisms, which is probably dependent on the very here heterogeneous environment including diverse niches and milieus in the CF lung [5].

References

[1] Römling U. et al. 2013. *Microbiol Mol Biol*
[2] Blanka A. et al. 2015. *Sci Signal*
[3] Malone JG. et al. 2010. *PLoS Pathog*
[4] Häußler S. et al. 2003. *J Med Microbiol*
[5] Winstanley C. et al. 2016. *Trends Microbiol*

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

648/KMP

***In vivo* transcriptional profiling of *Pseudomonas aeruginosa* clinical isolates derived from transplanted lung tissue**

A. Kordes*[1], M. Schniederjans[2], U. Muthukumarasamy[2], P. Braubach[3], S. Häußler[2,1]
[1]*Institute for Molecular Bacteriology, TWINCORE, Centre for Experimental and Clinical Infection Research GmbH, Hannover, Germany*
[2]*Department of Molecular Bacteriology, Helmholtz Center for Infection Research, Braunschweig, Germany*
[3]*Institut für Pathologie, Medizinische Hochschule Hannover, OE 5110, Hannover, Germany*

Introduction: *Pseudomonas aeruginosa* is a human pathogen associated with several hospital acquired diseases, e.g. acute infections of burn wounds or chronic infections of lungs in patients suffering from cystic fibrosis (CF). An emerging challenge is the antibiotic resistance and the formation of biofilm making it difficult to treat the patients. During the switch towards a chronic infection *P. aeruginosa* undergoes several adaption processes, e.g. switch to a sessile lifestyle, loss of virulence or increased antibiotic resistance [1]. Various genomic and transcriptomic studies with sputum samples from CF patients revealed a set of acquired pathoadaptive mutations and important genes expressed in the CF lung [2,3]. But still, not much is known about the *in vivo* transcriptional profile of *P. aeruginosa* in the CF lung.
Objectives: In this study we aim to characterize the *in vivo* transcriptional profile of *P. aeruginosa* adapted to the lung of patients suffering from CF. Analyzing the *in vivo* transcriptional profile of several clinical isolates derived from transplanted lungs could give new insights of *P. aerugionsas* lifestyle *in vivo*.
Materials & methods: The left lobe of a CF lung was cutted into small pieces directly after lung transplantation. RNA was extracted from tissue samples derived from five different locations of the lung (Bronchus, central and peripheric part of the upper and inferior lobe respectively). The eukaryotic mRNA was depleted and remaining prokaryotic mRNA was deep sequenced with Illumina. Optained reads were mapped against *P. aeruginosa* PA14 wild type to analyze the expression profile.
Results: After establishing and improving our protocols we were able to sequence the prokaryotic mRNA out of the CF lung from three different patients. Only the samples from one patient resulted in a sufficient number of reads (30 billion reads in total), but still over 80% of the reads were eukaryotic. Nevertheless we could identify more than 1.5 billion reads mapping to *P. aeruginosa*. Comparing our data with the transcriptional profile of the lab strain PA14 grown under normal LB-conditions or PA14 derived from an *in vivo* tumor model revealed huge differences. However, we could not see large differences in the transcriptional profile between the strains obtained from the five locations of the lung.
Conclusion: It was possible to analyze the *in vivo* transcriptional profile of a *P. aeruginosa* clinical isolate which adapted to the cystic fibrosis lung. To further investigate if there is a common or strain specific transcriptional profile of *P. aeruginosa in vivo* we want to sequence more samples from different patients.

References

[1] Damkiær et al. 2013, *Proc Natl Acad Sci USA*
[2] Marvig et al. 2015, *Nature Genetics*
[3] Bielecki et al. 2012, *Environ Microbiol*

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

649/KMP

Genotype-phenotype correlation studies of structure-related clinical *Pseudomonas aeruginosa* isolates

J. G. Thöming*[1], M. Schniederjans[2], A. Kordes[1], M. Müsken[1,2], S. Häußler[1,2]
[1]*TWINCORE, Centre for Experimental and Clinical Infection Research, Molecular Bacteriology, Hannover, Germany*
[2]*Helmholtz Centre for Infection Research, Department of Molecular Bacteriology, Braunschweig, Germany*

Introduction: The opportunistic pathogen *Pseudomonas aeruginosa* belongs to one of the most clinically relevant organisms and is a leading cause for morbidity and mortality especially in the chronic lung infection of cystic fibrosis patients [1]. Due to its capability to form biofilms, *P. aeruginosa* is extremely difficult to eradicate since the embedment in a self-produced extracellular matrix enables a protective environment and results in enhanced tolerance towards antimicrobial treatment as well as the host immune response [2,3]. Whether certain clinical isolates do share characteristic biofilm features, which result in a similar response, is, however, not yet understood.
Objectives: In this study, we aim to determine biofilm phenotypes of clinical isolates and cluster them according to structural features to further investigate whether the isolates share additional features. Moreover, genotype-phenotype correlation studies are applied to uncover and predict clinically relevant properties.
Materials & methods: A high-content screening of >500 clinical isolates was performed by using a microtiter plate-based *in vitro* biofilm assay in combination with automated confocal laser scanning microscopy (CLSM). Isolates were grouped according to their biofilm structure and selected strains of each cluster were further characterized with regard to the phenotypes: colony morphology, antibiotic resistance profiles (five different classes) and virulence (*Galleria mellonella* infection model). Moreover, transcriptional profiles displaying the planktonic (P) as well as biofilm (BF) state were generated and analyzed.
Results: In our screening, three major biofilm clusters were identified: unstructured biofilms with a high biomass (C1), biofilms showing a fine and filamentous network (C2) and small microcolony-like aggregates (C3). While colony morphology and antibiotic resistance do not correlate with a particular biofilm cluster, there seems to be a difference in the *Galleria* model. Isolates of cluster C1 are mainly virulent in contrast to the avirulent isolates of cluster C2. The analysis of transcriptional profiles (BF) shows that the respective isolates of the three groups cluster together. Thereby, the Type 3 secretion system (T3SS) as well as genes related to motility and attachment (e.g. type IV pilus) are differentially expressed in at least one cluster compared to the others.
Conclusion: Our work reveals that the biofilm structure has no direct impact on the antibiotic resistance whereas a correlation with virulence could be shown. The generated transcriptome data already shows specific expression profiles but needs to be further investigated (planktonic vs. biofilm) to determine biofilm- and isolate-specific adaptation mechanisms (e.g. vs. antibiotics) and to unravel the clinical role of biofilm structures in chronic infections.

References

[1] Govan & Deretic (1996), *Microbiol. Rev.*, vol. 60 no. 3 539-574
[2] Costerton *et al.* (1995), *Annu. Rev. Microbiol.*, doi: 10.1146/annurev.mi.49.100195.003431
[3] Moskowitz *et al.* (2004), *J. Bacteriol*., doi: 10.1128/JCM.42.5.1915-1922.2004

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

650/KMP

Supplementation of L-amino acids in excess influences *Pseudomonas aeruginosa* swimming and swarming motility

M. Donnert*[1], S. Haeussler [1]
[1]*Twincore - Centre for Experimental and Clinical Infection Research, Institute for Molecular Bacteriology, Hannover, Germany*

Introduction: *Pseudomonas aeruginosa* is found in various natural habitats such as soil and water. Moreover, it is an important opportunistic pathogen and the leading cause for lung infections in cystic fibrosis patients. *P. aeruginosa* is equipped with a polar flagellum and type IV pili which allows different locomotion such as swimming, swarming and twitching. It possesses a large set of virulence factors to cause acute and chronic infections and one of its striking features is its ability to switch between a motile and sessile lifestyle, the latter which is characterised by a biofilm mode of growth in which the bacteria are protected against antibiotics. Apart from intracellular signals, the transition between these two stages is also triggered by environmental cues like temperature, cell density and nutrient availability. Recently, Bernier *et al.* (2013)1 showed that upon addition of several L-amino acids, especially arginine was able to enhance biofilm formation in *P. aeruginosa* and at the same time inhibiting swarming motility, assuming a role for arginine in regulating the switch between motility and biofilm formation.
Objectives: The aim of our study is to investigate in detail the influence of amino acids on *P. aeruginosa* motility behaviour with a special focus on swimming and swarming. The project aims at i) finding distinct amino acids that are able to enhance motility in order to e.g. promote dispersal in biofilms, hence increasing the efficiency of antibiotic treatment and ii) with promising amino acids in hand, studying their possible mode of action to better understand the underlying regulatory mechanisms.
Materials & Methods: For this purpose, *in vitro* motility assays on semi-solid surfaces were adapted from Ha *et al.* (2014)2 and applied to screen the standard laboratory strain PA14. The experimental set-up was optimised for testing each of the 20 proteinogenic L-amino acids, using BM2 minimal medium supplemented with the respective amino acid in 10 mM excess. Furthermore, microscopic techniques and data from RNA-sequencing and ribosome profiling should be implied to account for possible mechanisms.
Results & Conclusion: From the 20 amino acids tested, asparagine and lysine where shown to increase swimming behaviour, whereas a decrease was observed especially upon addition of glutamine and serine. The latter was also found to clearly inhibit swarming, along with 4 other amino acids including arginine. In contrast, swarming was enhanced by a total of 5 amino acids with the highest increase seen for isoleucine and methionine. These effects were not correlated to differences in growth dynamics as determined by planktonic growth, indicating a possible regulatory role which has to be further investigated by ongoing experiments – with Asp, Lys, Ser and Arg as the most promising amino acids for future studies.

References

[1] DOI: 10.1016/j.resmic.2011.04.014
[2] DOI: 10.1007/978-1-4939-0473-0_7

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

651/KMP

Knowledge update - Antimicrobial prophylaxis in joint replacement procedures: need to change current practice?

C. Berberich*[1]
[1]*Heraeus-Medical, Medical Training, Wehrheim, Germany*

The implementation of routine perioperative antibiotic prophylaxis (PAP) in surgical procedures involving the implantation of foreign material is undoubtedly one of the most effective factors in reducing the number of superficial and deep surgical site infections (SSI). However, guideline recommendations on PAP in arthroplasty are relatively old and date back to studies of the 80ies and 90ies. No unanimous consensus on the "best" prophylactic drug exists. Cephalosporines of 1.& 2. generation are still appropriate and most frequently applied in most hospitals, but PAP should be critically reviewed in institutions with overall high infection rates and/or high prevalence of MRSA/MRSE or gram-negative bacteria as cause for SSI. A trend to a dual, but often varying PAP (i.e. glycopeptide plus aminoglycoside/ cephalosporin) is visible in some institutions (in particular in UK). While an additional prophylactic effect has been demonstrated in some institutions for a dual PAP strategy, others have failed to show a clear clincial benefit. In the best case local resistance surveillance data should guide PAP decisions. "Customized PAP" may also be considered in patients at particular high risk of infections and in special situations (2nd stage of septic revisions, patients with symptomatic bacteriuria in acute trauma interventions etc.). Local antibiotics establish an additional "battle line" against contaminating bacteria directly at the most vulnerable site in joint replacement procedures. Prophylactic efficacy of antibiotic-loaded bone cement has just been re-confirmed in the fractured hip infection trial in the UK where a combination of local high dose gentamicin & clindamycin (1 g each per 40 g of cement) significantly reduced the deep infection number in this frail patient population from 3.5% to 1,1%.

Figure 1

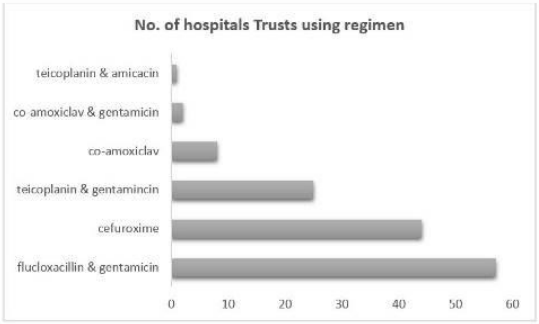


Fig. 1. Overview on some prophylactic antibiotic regimen in use for patients undergoing hip and knee arthroplasty according to a survey among Trust hospitals in UK (Graph adapted according to Hickson et al. 2015)

Figure 2

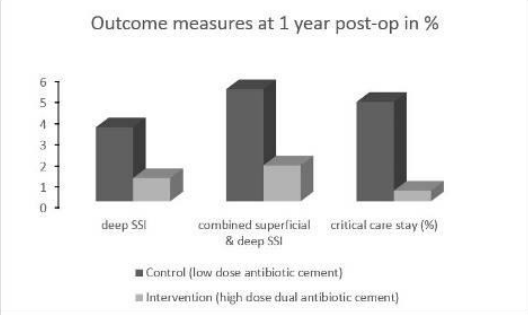


Fig. 2. Results from a clinical trial in Northumbria Trust hospitals in UK: 848 patients with intracapsular neck of femur fractures were randomized to receive either high dose dual antibiotic loaded cement COPAL G+C (1 g gentamincin & 1 g clindamycin per 40 g of cement powder) or the standard low dose single antibiotic cement PALACOS R+G (0,5 g gentamicin per 40 g of cement powder). Patients were followed up during an observation period of 1 year (adapted according to Sprowson et al., 2016).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

652/KMP

Potentiality of Lytic Bacteriophages and their Virolysins in Lysing Multi-Drug Resistant *Salmonella typhi*

A. Elshayeb*[1], A. Ahmed[1], M. El Siddig[1], A. EL Hussien[1]
[1]*University of Khartoum, Faculty of Science, Khartoum, Sudan*

Bacteriophage virolysins or lytic enzymes are bacterial peptidoglycan hydrolases responsible for the lysing of bacterial cells. Consequently can be used as Enzybiotics alongside with the bacteriophage therapy in remedying multi-drug resistant *Salmonella typhi*. To evaluate the potentiality of virolysins on curing multi-drug resistant *S. typhi,* both microorganisms were isolated and identified according to World Health Organization and International Standardization Organization guidelines. The antibiotics susceptibilities were tested using the Clinical Laboratory Standards Institute recommendations. Correspondingly, the bacteriophage-lysing efficiency was assayed by double-layer agar technique. The virolysins were extracted using ultracentrifugation and purified by dialysis after buffering in ammonium sulfate. The enzymes activity was determined by measuring the reducing sugars released from lysed *S. typhi* in the reaction mixture spectrophotometrically at Optical Density (OD600nm) where bacteria incubated as substrate in 37oC for 4 hours. The phages and their virolysins kinetics exponential rates were calculated using specific differential equations. Susceptibility plotted data on antibiogram criteria confirmed that 33% of *S. typhi* isolates were multi-drug resistant. For bacteriophage replication and the multiplicity of infection, *S. typhi* corresponding phages were amplified to produce the maximum phage particles of high titers. The phage titration fitting data on sensogram revealed exponential decay of *Salmonella typhi* in 12 hours of incubation. Meanwhile, solving the enzyme kinetics exponential decay on double reciprocal plot showed irretrievable relationship of host decay in 4 hours of incubation. Since phages depend on their lytic cycle in lysing bacterial host, their enzymes have more capability in decaying the host, beside they are safe and time-saving when used in the treatment of antibiotics resistant *S. typhi*.

References

Elshayeb A.A. et al, (2016). Potentiality of Lytic Bacteriophages and their Virolysins in Lysing Multi-Drug Resistant Salmonella typhi. Submitted

Figure 1: Virolysins Kinetics exponential decay

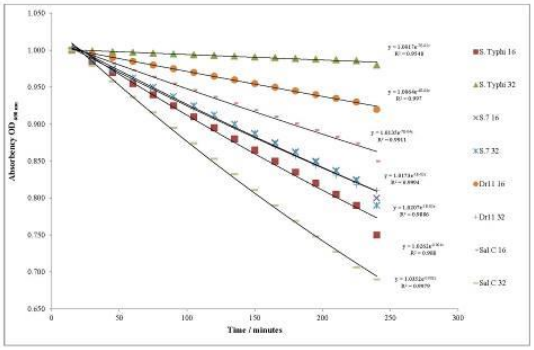


Figure 4: Virolysins Kinetics exponential decay:

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

653/KMP

The antimicrobial compound Gentisaldehyde - a potential agent to prevent bovine mastitis

A. Schabauer*[1], C. Zutz[2], G. Mößlacher[3], K. Rychli[1], M. Wagner[1]
[1]*Institute of Milk Hygiene, Milk Technology and Food Science , University of Veterinary Medicine Vienna, Department for Farm Animal and Public Health in Veterinary Medicine, Vienna, Austria*
[2]*Research Platform Bioactive Microbial Metabolites (BiMM), Bioresources and Technologies Campus Tulln, Tulln an der Donau, Austria*
[3]*Upper Austrian Animal Health Service Laboratory, Ried im Innkreis, Austria*

Introduction: *Staphylococcus (S.) aureus* is known to be the most common contagious pathogen causing udder infections in cows. Significant economic loss and frequent use of antibiotic agents are the results of mastitis in dairy industries. Therefore, attention and concern on occurring antibiotic resistance and its negative effects are growing.

Objectives: This study focuses on resistance of mastitis isolates to commonly used biocides (antibiotics, disinfectants) and on the development and introduction of alternative antimicrobial compounds for treatment and prevention of mastitis.

Materials & methods: A diverse set of 184 isolated *Staphylococcus aureus* mastitis strains, including 54 different and six new *spa*-genotypes, was designed. The antimicrobial activity and cytotoxicity of the underresearched antimicrobial substance "Gentisaldehyde", isolated from a fungus in a previous project, have been invastigated. Furthermore, the minimum inhibitory concentrations of three commonly used disinfectants (Benzalkonium chloride, Chlorhexidine and Iodine) have been determined. Additionally, resistance to a panel of selected antibiotic agents was analysed by disk diffusion method according to EUCAST 2015/2016.

Results: Antibiotic resistance to Benzylpenicillin was detected in 29 % isolates, Tetracycline in 4 %, Cefoxitin and in Erythromycin in 3 % of the *S. aureus* strains. Multi-resistance (defined as reduced susceptibility to more than three antibiotic classes) has been found in 2.7 % of isolates. The distribution of the disinfectants' minimum inhibitory concentrations was very homogenous: 91 % of isolates showed a MIC of 4 µg/ml for Benzalkonium chloride, 87 % showed a MIC of 2 µg/ml for Chlorhexidine and 94 % showed a MIC of 500-600 µg/ml for Iodine. Gentisaldehyde inhibited the growth of 87 % of isolates at a concentration of 500 µg/ml, including all multi-resistant strains. At this concentration, no cytotoxicity was observed using human hepatocytes HepG2 and human intestinal epithelial Caco2 cells. Further, cross-resistance to one of the other disinfectant

substances was not detected. In some isolates, a trend of increased sensitivity to both Benzalkonium chloride and Chlorhexidine was found.
Conclusion: The substance Gentisaldehyde efficiently inhibits the growth of a diverse set of *S. aureus* mastitis isolates, including multi-resistant strains, at non-cytotoxic concentrations. Therefore, it could be an effective compound of an antiseptic used for teat dipping in the prevention of bovine mastitis, leading to reduced use of antibiotics in dairy cows.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

654/KMP
Molecular microbiology as a modern platform for rapid, specific, sensitive and unlimited detection of pathogenic microorganisms
S. Pekova*[1,2]
[1]*KitGen s.r.o., Laboratory for molecular diagnostics, Prague, Czech Republic*
[2]*Vemodia a.s., Laboratory for veterinary molecular diagnostics, Prague, Czech Republic*

Molecular microbiology is a novel concept that opens up novel and fascinating possibilities in the pathogen detection. Given the fact that many microorganisms are fastidious or uncultivatable (e.g. strictly anaerobic bacteria, *Chlamydia*); their cultivation time is unacceptably long (e.g. *Mycobacterium*); are of high epidemiological importance (e.g. novel Influenza strains, emerging zoonoses) or require sophisticated cultivation conditions (e.g. many viruses), it is highly relevant to implement modern molecular techniques into the current guidelines for the laboratory diagnostics. Molecular techniques allow for quantitative detection of an unlimited number of microorganisms (bacteria, fungi, viruses, microscopic parasites), based solely on the presence of their unique DNA or RNA sequences. Molecular microbiology enables to identify causative infectious agents even in those situations when standard cultivation-based microbiology fails. Given the fact that the precise knowledge of the identity of the infectious agent is the absolute prerequisite for targeted and timely antimicrobial treatment, we strongly advocate molecular microbiology be an integral part of the modern management of infectious diseases.
Since 2006, we have developed over 500 single and multiplex quantitative Real-Time PCR assays to detect pathogenic and opportunistic infectious agents relevant for both human and veterinary clinical settings. We have also developed pandetection approach to detect bacteria and fungi based on Sanger sequencing. For the most challenging biological samples (stool, samples with primarily mixed microbial flora) we have also developed a pandetection strategy based on Next Generation Sequencing (NGS). Using this combined approach, we are now able to identify causative microbial agents with the widest detection range possible (pandetection), quantify the load of individual microorganisms in the sample and provide the clinician with the result within hours (Real-Time PCR), or 2-3 days maximum (Sanger sequencing or NGS).
Over the past 10 years we have diagnosed more than 30,000 biological samples, originating from both human and veterinary patients. They covered hyperacute clinical settings (sepsis, meningitis, severe pneumonias), chronic and underdiagnosed diseases (intraocular chlamydiasis, infectious endocarditis caused by rare pathogens, chronic pulmonary or urinary infections, patients with inflammatory bowel diseases), and emerging zoonoses (our finding of a novel zoonotic agent *Candidatus Neoehrlichia Mikurensis*, transmissible by a tick bite, with unexpected Central and Western European geographic occurrence).
The presented data will cover a unifying concept of molecular microbiology, covering both human and veterinary infections, as despite the modern era, infectious diseases still top the list of mortality and morbidity causes worldwide.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

655/KMP
Expired nitric oxide and sputum mycobacterial lipid bodies indicate that pulmonary NO is a double edged sword in tuberculosis.
B. Gelaw*[1], N. Garton[1,2], A. Assefa[1,2,3], M. R. Barer[1,2,3]
[1]*University of Gondar, Medical Microbiology, Gondar, Ethiopia*
[2]*University of Leicester, Infection, Immunity and Inflammation, Leicester, United Kingdom*
[3]*AHRI, Molecular Biology, Addis Ababa, Ethiopia*

Background: The variable occurrence of lipid body rich (fat) and poorly replicating (lazy) *Mycobacterium tuberculosis* bacilli in sputum necessitates an explanation of the environmental signals responsible for these phenotypes. Lipid body rich and poorly replicating *Mycobacterium tuberculosis* bacilli occur at different frequencies in sputum. In vitro, NO stimulates lipid body (LB) accumulation in *M. tuberculosis* via the dormancy-associated regulon [DosR (DevR)]. We hypothesized that the percentage of lipid body-positive acid fast bacilli (%LB+AFB) in sputum correlates with fractional expired NO (FeNO) and that greater LB responses to NO might be associated with poorer responses to chemotherapy.
Methods: In Gondar, Ethiopia, 73 patients with smear positive tuberculosis were recruited and assessed for sputum %LB+AFB, FeNO and HIV status. Weight gain was determined at 7 months in 9 patients as a measure of treatment response.
Results: %LB+AFB in patients" sputum significantly associated with Log10FeNO concentration ($p<0.001$) with a linear relationship ($r2 = 0.209$, $p<0.001$). Weight gain showed a negative linear association with %LB+AFB at both 2 ($r2=0.196$) and 7 months ($r2=0.445$) of treatment. Stronger correlations of Log10FeNO concentration with %LB+AFB were apparent after stratification for HIV status with a shallower negative gradient for HIV positives.
Conclusions: *M. tuberculosis* LB frequencies in sputum are significantly associated with patient FeNO levels in a manner consistent with bacterial DosR activation by NO in the lung. DosR activation is associated with antibiotic tolerance and may compromise treatment response while bactericidal effects of NO should be beneficial. We suggest that NO is a double-edged sword enabling mycobacterial clearance at high levels but provoking antibiotic tolerance when sub-lethal.
Keywords: Tuberculosis; expired NO; mycobacterial lipid bodies; DosR regulon, treatment response.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

656/KMP
Emerging Antimicrobial Resistance Due to Misuse of Antibiotics: A Study Conducted Among The University Students in Pakistan
Z. Rahman*[1], M. Ullah[1]
[1]*Abdul Wali Khan University Mardan, Microbiology, Mardan, Pakistan*

The antimicrobial resistance (AMR) is a global issue due to emergence of pathogenic strains. The major problems in antimicrobial resistance is the misuse of antibiotics. Here, we investigate about the awareness for proper use of antibiotics among the students of Abdul Wali Khan University Mardan, Pakistan. Undergraduate and graduate students were interviewed through questionnaires. The main questions were related to the use of antibiotics during their illness period. As a result, during a fever of 100oC or above, 42% students were like to take

antibiotics without doctors prescription. The antibiotics preferred to take without the prescription were amoxicillin, cephalosporin, and erythromycin by 15, 19 and 54% students, respectively. Almost 100% of students knew about the AMR, still 67% preferred to buy antibiotics from medical stores without doctors prescription. The students were not having any medical insurance and they had to visit private hospitals and clinics compared to that of the government due to lack of proper management. The results of the study showed that due to lack of health insurance, proper awareness of antibiotic use, and selling of antibiotics without standard procedures recommended by Drug Regulatory Authority of Pakistan, the AMR could further favor the emergence of antibiotic resistance. We recommend the government to take measures and to educate people for the proper use of antibiotics. Furthermore, there should be check and balance over the medical stores in Pakistan for sale of antibiotics according to standard procedures.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

657/KMP

Isolation of spontaneous Lipopolysaccharide mutants from *Coxiella burnetii*

K. Mertens*[1], F. Csicsay[2], L. Skultety[2], C. Gerlach[1], K. Henning[1], H. Neubauer[1]

[1]*Friedrich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany*

[2]*Institute of Virology, Slovak Academy of Sciences, Department of Rickettsiology, Bratislava, Slovakia*

Coxiella burnetii is the etiological agent of query (Q) fever. This zoonotic disease is worldwide distributed and ruminants are considered as the main reservoir for human infections. Q fever manifests as an acute flu-like illness or as chronic disease. The latter can be life-threatening and has a high impact on the public health system. There is no licensed vaccine available for humans. The current used vaccine for ruminants reduces abortions and shedding but does not eliminate the agent.

The Lipopolysaccharide (LPS) is associated with a protective immune response and plays a major role during receptor-mediated phagocytosis of *C. burnetii*. Upon serial passage *C. burnetii* undergoes a phase variation where virulent phase I (Ph I) convert to avirulent phase II (Ph II). Ph I organisms express a smooth LPS, while ph II express a rough LPS. Vaccination with inactivated *C. burnetii* phase I has been shown to be protective in a guinea pig infection model. The major difference between Ph I and Ph II bacteria is the LPS molecule and indicates that Ph I LPS plays a critical role in the development of protective immunity against *C. burnetii* infection.

The underlying mechanism of phase variation has not been identified yet. There are several known Ph II strains, some with chromosomal deletions in regions encoding for sugar processing enzymes and polysaccharide transporters, but with different LPS chemotypes. Other known Ph II strains carry no deletion within this specific region and it is likely that one or more additional mutations may be responsible for loss of the OPS.

In this study we attempt to characterize the mechanism of phase variation by correlation of structural data and genomic information from spontaneous LPS mutants. Therefore we are using two different strategies for generation of spontaneous LPS mutants. We are serially passaging the Nine Mile Ph I strain in axenic medium and we use the egg passage 41 (EP41) of *C. burnetii* St 48. After propagation in axenic medium cultures are plated on semi-solid medium for clonal isolation. SDS-PAGE and silver straining of crude LPS extracts is used to monitor passages and clones for changes of LPS banding patterns. Based on the detected LPS chemotype clones are tested for mutations or deletions by amplification and sequencing of the corresponding gene clusters. Detailed structural analyses of the LPS (sugar composition and linkage) will be performed on selected clones to identify major steps of the LPS biosynthetic pathway and to understand the phase variation phenomenon of *C. burnetii*.

The Nine Mile Ph I strain was passaged 12 times over 17 weeks in axenic medium. From this strain we could isolate a subpopulation of Ph II bacteria, expressing a rough LPS. They show a smaller deletion of approximately 20 kbp within the OPS-encoding gene cluster, when compared to the 26 kbp deletion of Nine Mile Ph II. The deletion site in both Ph II strains was determined by sequencing and the LPS banding patterns displayed visible changes. Clonal isolation and characterization of *C. burnetii* St 48 EP41 LPS mutants are still ongoing. We will further use Nine Mile phase I for egg passaging to generate mutants with different LPS chemotypes than Ph II.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

658/KMP

Nitazoxanide and Doxycycline Sensitivity among Metronidazole Resistant *Helicobacter pylori* Isolates from Patients with Gastritis

A. Baradaran Moghaddam*[1], N. Amirmozafari[1], M. Alebouyeh[2]

[1]*Iran University of Medical Sciences, Microbiology, Tehran, Islamic Republic Of Iran*

[2]*Shahid Beheshti University of Medical Sciences, Gastroenterology and Liver Diseases, Tehran, Islamic Republic Of Iran*

Question: *H. pylori* is one of the most common pathogens that causes chronic inflammation of the gastrointestinal tract in humans and is an important risk factor in chronic gastritis, gastric ulcer, duodenal ulcer and gastric cancer (1). *H. pylori* antibiotic resistance is the main factor affecting the effectiveness of current therapeutic regimens (2-3). According to the high prevalence of *H. pylori* infection and its complications and increasing resistance to metronidazole that cause treatment failure, we examined antibacterial activity of nitazoxanide and doxycycline against the clinical *H. pylori* isolates showing different metronidazole resistance levels.

Methods: A total of 122 patients from three hospitals who underwent endoscopy were enrolled in this study from three hospitals in Tehran, during November 2014 to July 2015 in Tehran, Iran. *H. pylori* isolates were obtained from gastric biopsies of the patients after culture in specific culture medium and characterization by both biochemical and molecular methods. Antimicrobial susceptibility of the strains to metronidazole was detected using agar dilution method and minimum inhibitory concentrations of nitazoxanide and doxycycline were determined for the metronidazole resistant strains.

Results: From a total of 122 gastric biopsy specimens, 55 *H. pylori* strains were recovered (45%). Thirty three of these strains (60%) were resistance to metronidazole. MIC50 and MIC90 values for metronidazole were 32 and 64 µg/ml, respectively. MIC50 and MIC90 values for doxycycline and nitazoxanide were measured as 4 and ≥8 µg/ml, and 8 and ≥32 µg/ml, respectively.

Conclusions: Dominance of high level of metronidazole resistance *H. pylori* strains among the studied patients questioned its usefulness for first-line therapy in Iran. Nitazoxanide and doxycycline showed superior activity against *H. pylori* strains in comparison to metronidazole, which should be considered for alternative therapies.

References

[1] Wotherspoon MR, Path AC. Gastric lymphoma of mucosa-associated lymphoid tissue and Helicobacter pylori. Annual Review of Medicine. 1998; 49(1):289–99.

[2] Wu H, Shi X, Wang H, Liu J. Resistance of Helicobacter pylori to metronidazole, tetracycline and amoxycillin. Journal of Antimicrobial Chemotherapy. 2000; 46(1):121–3

[3] Khedmat H, Karbasi-Afshar R, Agah S, Taheri S. Helicobacter pylori Infection in the general population: A Middle Eastern perspective. Caspian Journal of Internal Medicine. 2013; 4(4):745–53

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

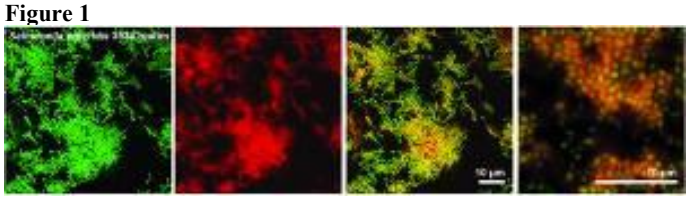
659/KMP

Real-time optotracing of curli and cellulose in live *Salmonella* biofilms using luminescent oligothiophenes

F. X. Choong*[1], M. Bäck[2], K. Melican[1], M. Rhen[1], K. P. R. Nilsson[2], A. Richter-Dahlfors[1]
[1]*Karolinska Institutet, Stockholm, Sweden*
[2]*Linköping University, Linköping, Sweden*

Extracellular matrix (ECM) is the protein- and polysaccharide-rich backbone of bacterial biofilms that provides a defensive barrier in clinical, environmental and industrial settings. Understanding the dynamics of biofilm formation in native environments has been hindered by a lack of research tools. We have developed a method for simultaneous, real-time, *in situ* detection and differentiation of the *Salmonella* ECM components curli and cellulose, using non-toxic, luminescent conjugated oligothiophenes (LCOs). These flexible conjugated polymers emit a conformation-dependent fluorescence spectrum, which we use to kinetically define extracellular appearance of curli fibers and cellulose polysaccharides during bacterial growth. The scope of this technique is demonstrated by defining biofilm morphotypes of *Salmonella enterica* serovars Enteritidis and Typhimurium, and their isogenic mutants in liquid culture and on solid media, and by visualizing the ECM components in native biofilms. Our reported use of LCOs across a number of platforms, including intracellular cellulose production in eukaryotic cells and in infected tissues, demonstrates the versatility of this optotracing technology, and its ability to redefine biofilm research.

Figure 1



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

660/KMP

Emergence of ceftazidime-avibactam non-susceptibility in a multidrug resistant *Klebsiella pneumoniae* isolate

A. Both*[1], C. Belmar Campos[1], M. Christner[1], F. Maurer[1], H. Büttner[1], C. König[1], M. Aepfelbacher[1], D. Wichmann[1], H. Rohde[1]
[1]*UK Hamburg-Eppendorf, Hamburg, Germany*

Avibactam is a novel broad-range beta lactamase inhibitor active against Ambler class A- (including ESBL and KPC) and some Ambler class D- (e.g. OXA-48) enzymes. We here report on the emergence of ceftazidime-avibactam resistance in clinical multi-resistant, OXA-48 and CTX-M-14-producing *K. pneumoniae* strain DT during ceftazidime-avibactam treatment. Comparative whole genome sequence analysis identified two single nucleotide polymorphisms in the CTX-M-14-encoding gene leading to two amino acid changes. Compared to wild type CTX-M-14, expression of CTX-M-14ΔΔ isoform in *E. coli* Top10 let to a 1024- and 16-fold increase in ceftazidime and ceftazidime-avibactam MICs, respectively, functionally linking the observed SNP and elevated MICs. The mutated CTX-M-14 isoform exhibited augmented ceftazidime hydrolytic activity, being a reasonable cause for impaired efficiency of avibactam inhibition. CTX-M-14ΔΔ was associated with elevated ceftazidime-avibactam MICs in independent *K. pneumoniae* isolates, but was not sufficient for full resistance. Apparently, additional, CTX-M-independent mechanisms contribute to ceftazidime-avibactam resistance in *K. pneumoniae* DT. This first study on the molecular basis of ceftazidime-avibactam-resistance in *K. pneumoniae* emerging *in vivo* underscores the need for continuous monitoring of ceftazidime-avibactam-susceptibility during therapy. Rapid development of CTX-M-14 isoforms exhibiting augmented ceftazidime hydrolytic activity may limit usefulness of ceftazidime-avibactam monotherapies in infections caused by isolates carrying both, *bla*CTX-M-14 and *bla*OXA-48.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

661/KMP

Drug repurposing studies on existing US FDA approved drugs for intervention against Tuberculosis

A. Kumar*[1], A. Alam[1], S. Grover[1], N. Z. Ehtesham[2], S. E. Hasnain[1,3]
[1]*Indian Institute of Technology Delhi, Kusuma School of Biological Sciences, New Delhi, India*
[2]*National Institute of Pathology, Safdarjang Hospital Campus, New Delhi, India*
[3]*Jamia Hamdard, JH-Institute of Molecular Medicine, New Delhi, India*

Tuberculosis caused by *Mycobacterium tuberculosis*, takes one human life every 15-20 seconds globally. While there is multi drug therapy for TB, this involves taking a combination of four drugs for a period of 6-9 months, often causing toxicity to the patients. The prolonged treatment is due to the ability of this slow growing bacterium to trigger several host cell processes and form a biofilm – a sort of protective layer around it making bacteria drug tolerant. We have identified two US FDA approved drugs that inhibit biofilm formation that leads to increased vulnerability of bacteria to antibiotics resulting in more effective and faster killing of bacteria by drugs. Interestingly, there was no effect of similar concentration of these drugs on the growth of Mycobacteria present in planktonic stage that avert possibility of development of drug resistance. We carried out the interaction study of these drugs and found that it interacts with one of the Mycobacterial cyclophilins that induces the biofilm formation when overexpressed *in vivo*. Our results identified potential candidate drugs against TB, including MDR/XDR based on drug re-purposing acting via regulating biofilm formation. We further suggest that these new US FDA approved drugs for other diseases, may also lead to increased drug accessibility with likely impact on reducing treatment duration and overall cost of treatment. We believe that our strategy will also lead to a possible generic mechanism for combating other biofilm producing infectious bacteria.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

662/KMP

Bacterial-derived cell-penetrating peptides deliver gentamicin to kill intracellular pathogens

C. Rüter*[1], M. Gomaraska[1], T. Martins[1], L. Greune[1], P. Hardwidge[2], A. Schmidt[1]
[1]*ZMBE, Institute of Infectiology, Münster, Germany*
[2]*Kansas State University, Manhattan, Kasas, United States*

Commonly used antimicrobials show poor cellular uptake and often have limited access to intracellular targets, resulting in low antimicrobial activity against intracellular pathogens. An efficient delivery system to transport these drugs to the intracellular site of action is needed. Cell-penetrating peptides (CPPs) mediate the internalization of biologically active molecules into the cytoplasm. Here we characterized two CPPs, α1H and α2H,

derived from the Y. enterocolitica YopM effector protein. These CPPs, as well as Tat, were used to deliver the antibiotic gentamicin to target intracellular bacteria. The YopM-derived CPPs penetrated different endothelial and epithelial cells to the same extent as Tat. CPPs were covalently conjugated to gentamicin and CPP-gentamicin conjugates were used to target infected cells to kill multiple intracellular Gram-negative pathogenic bacteria, such as E. coli K1 RS218, Salmonella enterica serovar Typhimurium, and Shigella flexneri. Taken together, CPPs show great potential as delivery vehicles for antimicrobial agents and could contribute to the generation of new therapeutic tools to treat infectious diseases involving intracellular pathogens.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

663/KMP

Newer molecular targets and therapeutic strategies for intervention against *Mycobacterium tuberculosis*

S. E. Hasnain*[1,2], N. Z. Ehtesham*[3]
[1]*Indian Institute of Technology Delhi, Kusuma School of Biological Sciences, New Delhi, India*
[2]*Jamia Hamdard, JH-Institute of Molecular Medicine, New Delhi, India*
[3]*National Institute of Pathology, Safdarjang Hospital Campus, New Delhi, India*

Tuberculosis (TB) caused by *Mycobacterium tuberculosis (M.tb)*, takes one human life every 15-20 seconds globally. We have been focusing on the functional biology of this pathogen with a view to design innovative interventions against TB. We identified and characterized several virulent proteins of *M.tb* that help in intracellular survival by modifying host cellular machinery. Phylogenetic analysis of *M.tb* methyltransferases (MTases) pointed to an evolutionary relationship of *M.tb* with halotolerant organisms, notably in the context of their ability to withstand the host osmotic stress, thus highlighting their likely role in pathogenesis, virulence and niche adaptation. Some of the MTases exhibit antigenic patches and regulate transmembrane transport proteins. Another class of proteins, the sigma factors and there target genes, have been shown to move from non-pathogenic to pathogenic Mycobacteria.
The *M.tb* PE_PGRS subfamily has unusually high levels of disordered stretches compared to any other family in the proteome and was highly enriched in average number of ANCHOR binding sites, eukaryotic linear motifs (ELMs) and has highly biased amino acid composition rich in disorder promoting alanine and glycine residues and play roles in molecular mimicry. One member of this protein family causes activation of Unfolded Protein Response as evident from increased expression of GRP78/GRP94 and CHOP/ATF4, leading to disruption of intracellular Ca^{2+} homeostasis and increased NO and ROS production. The consequent activation of effector caspase-8, resulted in apoptosis of macrophages.
In other series of investigations, comparative proteomic and genomic analyses revealed the exclusive presence of "Signature sequences" in *M.tb* genome, some of which have potential utility in TB diagnosis based on limited clinical validation. Hypothetical proteins coded by one such "Signature sequences" was found to be a functional S-adenosyl dependent DNA methyltransferase and binds DNA non-specifically and protects DNA from oxidative stress by scavanging iron thereby, preventing generation of free radicals and also by physically binding DNA and providing a physical barrier.
Using drug re-purposing strategies we also identified existing US FDA approved drugs that inhibit *M.tb* by virtue of disrupting the pathogen"s biofilm forming ability and thus have the potential to act as a new TB drug and also to reduce the duration of treatment. My presentation will cover some of these findings from our group.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

664/KMP

Idiopathic Gingivostomatitis in Cats – an Underdiagnosed Ethiology

M. Naceradska*[1,2], S. Pekova[3]
[1]*Private Veterinary Practice, Prague, Czech Republic*
[2]*Veterinary and Pharmaceutical University Brno, Department of Infectious Diseases and Microbiology, Brno, Czech Republic*
[3]*Vemodia a.s., Laboratory for Veterinary Molecular Diagnostics, Prague 13, Czech Republic*

In a small animal veterinary practice, one of the most prevalent disorders of feline oral cavity is gingivitis / gingivostomatitis. The clinical presentation invariably involves irritated gums, with or without tissue lesions, poor dentition status and dental plaque of variable degree. Due to paucity of causative data on this clinical condition, the diagnosis is often inconclusive and the commonly used term idiopathic reflects the clinical desperation as to the pathogenesis of this clinical entity.
To test our hypothesis that the feline idiopathic gingivostomatitis is in reality an underdiagnosed clinical disorder of infectious origin we have systematically investigated oral cavity and tooth plaque swabs in a group of 25 cats using molecular microbiology techniques. Molecular microbiology, as it diagnostically relies on the DNA/RNA profiles of the microorganisms, can identify not only the most common microorganisms present in the oral cavity, but also fastidious and uncultivatable agents which are not detectable by classical microbiology and cultivation.
To reveal the full bacterial profiles of the oral cavity lesions in affected cats, primarily diagnosed as idiopathic gingivostomatitis, we have employed molecular pandetection of bacteria based on Sanger sequencing (SS) and Next Generation Sequencing (NGS) of evolutionarily conserved 16S rDNA genes, complemented with pathogen-specific Quantitative Real-Time PCR technology. Using this combined approach, we have identified many uncultivable/fastidious agents (*Porphyromonas gingivalis, P. endodontalis, P. canoris, Capnocytophaga canimorsus, Treponema denticola, T. putidum, Cardiobacterium spp., Filifactor spp., Peptostreptococcus micros, Actinomyces odontolyticus*, *Mycoplasma arginini, M. gateae and Chlamydophila felis),* many of which being highly suspected as causal agents of gingivostomatitis in man. Invariably, after targeted ATB treatment, remarkable improvement in the clinical status of the affected cats has been observed.
Thus, we conclude that the majority of idiopathic gingivostomatitis might in reality be a clinical condition of infectious origin caused by a microbial agent which classic microbiology fails to cultivate and thus, identify.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

665/KMP

Analysis of *Helicobacter pylori* strains from Colombian patients based on the presence of the protein CagA to determine the kind of infection.

D. F. Rojas- Rengifo*[1,2], L. F. Jiménez-Soto[2], J. F. Vera Chamorro[3], B. Molano[3], J. Alvarez[3], P. Rodríguez[3], L. C. Zuluaga[3], M. d. P. Delgado[1], R. Haas[2,4], C. A. Jaramillo[1]

[1]*Laboratiorio de Diagnostico Molecular y Bioinformática, Universidad de Los Andes, Bogotá, Colombia*

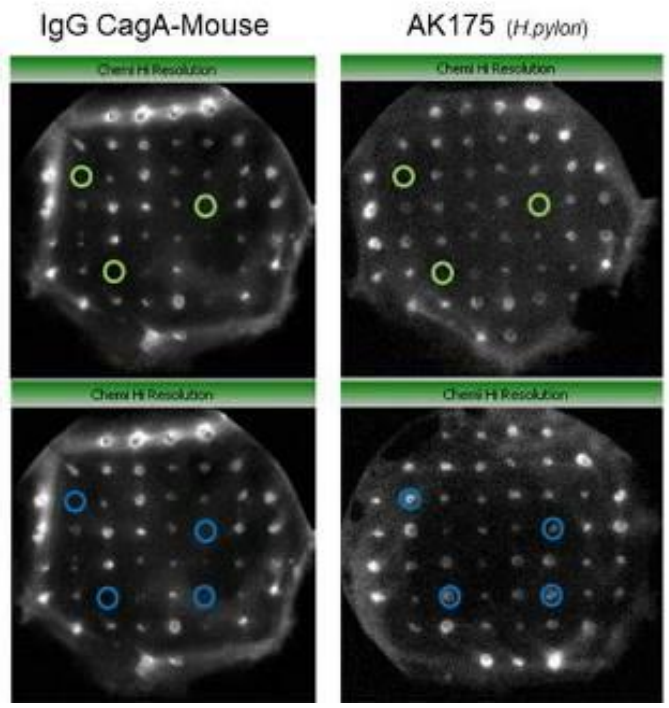
[2]*Max von Pettenkofer Intitut für Hygiene und Medizinische Mikrobiologie, Ludwig-Maximilians-Universität, München, Germany*

[3]*Hospital Universitario Fundación Santa Fe de Bogotá, Bogotá, Colombia*

[4]*German Center for Infection Research (DZIF) LMU München, München, Germany*

Helicobacter pylori is a gram-negative bacterium, which efficiently colonizes the human gastric mucosa. There are two types of strains classified by genotype. Type I contains the gene for CagA toxin and type II do not have it. Relative risk to present a gastric pathology increases by CagA positive strains. Colony Lift Immunoassay is a method to analyze and characterize isolated bacterial colonies. We have successfully adapted the assay for the detection of the CagA toxin production by *H. pylori* human isolates1. The aim of this study was to determine the prevalence of multiple infections with the *H.pylori* in Colombian patients. Patients older than 18 years presenting digestive symptoms were studied and an upper endoscopy was performed. Biopsies of the gastric antrum and corpus were taken. A culture of antrum and corpus"s biopsies was performed and up to 50 colonies from antrum and corpus were isolated and analyzed for CagA expression through a CLI with specific CagA antibodies, as recently published 1. A paired t-test and wilcox test were used considering a p value H. pylori (26.41%), 37 patients (43.52%) presented only type I strains while 48 (56.48%) showed mixed infections with *H. pylori* Type I and Type II strains. From these mixed infections, 26 (54.16%) patients showed strain specificity tropism for antrum or corpus colonization. There was not statistical significance of tropism for Type I strains (P=0.736), and the number of type I strains will be significantly higher than type II strains in patients with mixed infections (p= 4.565e-10). The data obtained using the CagA as marker for the type of strains present in the patients show a high prevalence of mixed infections in the population analyzed. Our data opens the possibility of a higher relevance and other implications of mixed infections in gastric pathology and should be considered not only in the evolution of pathological changes in the stomach associated with this bacteria, but as well in their possible relevance in the eradication therapies.

Figure 1



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

666/KMP

Lysis of bovine pericardial membranes for tissue grafting by bacterial pathogens causing infections in visceral surgery

M. Werner*[1], C. Bogdan[1]

[1]*Friedrich-Alexander-Universität (FAU) Erlangen-Nürnberg, Universitätsklinikum Erlangen, Mikrobiologisches Institut - Klinische Mikrobiologie, Immunologie und Hygiene, Erlangen, Germany*

Question: After implantation of collagen tissue meshes or patches in visceral surgery there were singular cases of infection with consecutive loss of the implanted membrane within few days, resulting in the necessity of operative revision. Aim of this study was to investigate, whether pathogens of local or systemic infections cause a significant reduction of the mechanical stability of bioscaffolds from bovine pericardium in vitro, and how this reduction progresses over time. Detection of gelatinase was tested for its suitability as an indicating parameter for imminent lysis of collagen membranes.

Methods: Samples of allografts from bovine pericardium (Tutomesh®, Tutopatch®, Tutogen Medical GmbH) were incubated with a defined inoculum of 55 different species of bacteria under standardized conditions. Membrane samples were checked daily for signs of occuring lysis for 14 days. By a second approach we quantified the reduction of ultimate tensile strength of the samples after 5 days of incubation utilizing Alluris FMT-310C5 Force Tester.

In order to determinate kinetics, samples of the same origin collagen membrane were incubated simultaneously with and without (control) bacterial inoculum. Measurement of the reduction of ultimate tensile strength was accomplished after 2, 4, 6, 8, 12, 16, 20, 24, 48 and 72 hours.

Detection of gelatinase was performed biochemically.

Results: *Proteus (P.) mirabilis, P. vulgaris, P. penneri, Serratia marcescens, Pseudomonas aeruginosa* and *Bacillus cereus* within a few days damaged Tutopatch® membranes intensely resulting in a massive loss of ultimate tensile strength up to total lysis.

There was a gradually inferior reduction of ultimate tensile strength at incubation with other pathogens. Incubation with *P. mirabilis* generated detectable damage already after 12 – 16 hours. The tensile strength expired totally after 24 – 36 hours.
Gelatinase was expressed even by bacteria species, which did not induce membrane lysis.
Conclusions: Certain pathogens of infections in visceral surgery are able to cause a reduction or loss of ultimate tensile strength of bioscaffolds from bovine pericardium right up to total lysis. Therefore the risk of suture insufficiency with necessity for operative revision is imminent. Detection of gelatinase does not allow a prognosis for possible membrane lysis.

Figure 1

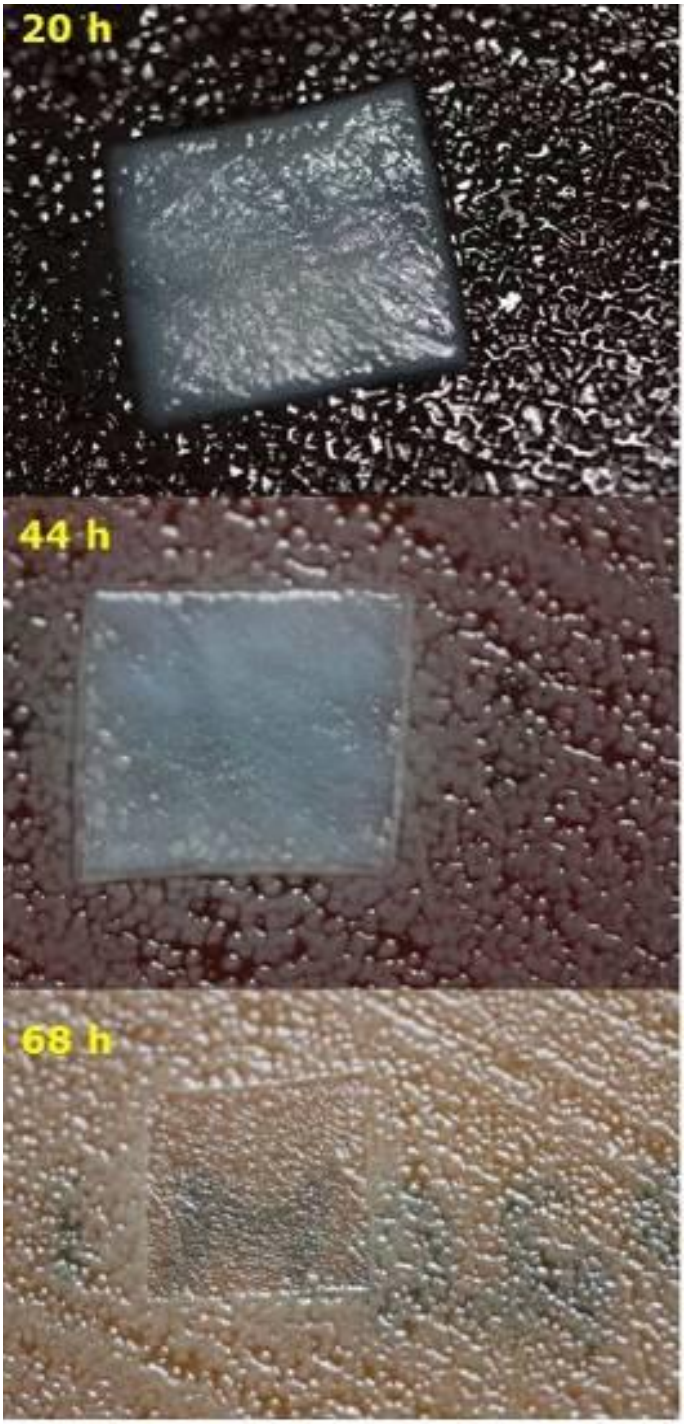


Fig.1) total lysis of collagen membrane Samples within 68 hours by *Proteus mirabilis*

Figure 2

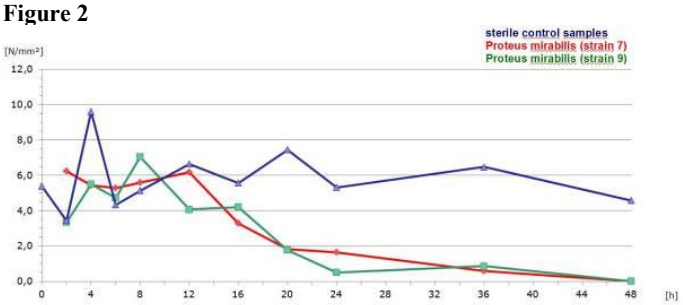


Fig. 2) kinetics of reduction of ultimate tensile strength by incubation with *Proteus mirabilis*

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

667/KMP

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections in children: experiences from a hospital in the Rhine-Main area

S. Albert-Braun[1], A. Weltzien[2], G. Werner[3], F. Layer*[3]
[1]*Klinikum Frankfurt Höchst, Institut für Laboratoriumsmedizin/ Krankenhaushygiene, Frankfurt am Main, Germany*
[2]*Klinikum Frankfurt Höchst, Klinik für Kinderchirurgie, Frankfurt am Main, Germany*
[3]*Robert Koch Institut , FG13 Nosocomial Pathogens and Antibiotic Resistance, Wernigerode, Germany*

Introduction: Since the 1990s CA-MRSA are described as pathogens emerging independent of health care. CA-MRSA are associated with the colonization of healthy, immunocompetent, younger individuals. They cause predominantly skin and soft tissue infections (SSTIs), but can also lead to life-threatening syndromes like necrotizing pneumonia and severe sepsis.
Objectives: The purpose of this retrospective study was the detailed investigation of stains isolated from community-onset MRSA-infections of children at hospital admission from 2012-2015 at the Klinikum Frankfurt Höchst.
Material & Methods: Strains were consecutively isolated from (community-onset) MRSA-infections of children at hospital admission. When possible, MRSA-screening of children and parents was also conducted. Initially, isolation of *S. aureus* and antibiotic susceptibility testing (AST) was performed at the hospital´s microbiology lab. The respective MRSA-strains were sent to the German NRC for Staphylococci and Enterococci for further characterization (AST by broth microdilution, SCC*mec*-typing, *spa*-typing, MLST, PCR-detection of marker genes associated with distinct CA-MRSA lineages). Medical records were reviewed to extract additional data, e.g. age, gender, clinical diagnosis, risk factors for acquisition of (CA-)MRSA and migration background. Furthermore data concerning other bacterial species received from abscesses treated at the pediatric surgery from 2012 to 2015 were studied retrospectively.
Results: In 42 cases an MRSA infection was diagnosed. 41/42 strains were clindamycin- and rifampicin-susceptible; 2/42 strains expressed cotrimoxazol-resistance. Two MRSA strains, which originated from ear-nose-throat-infections, showed characteristics of HA-MRSA (*spa*-type t003, t032). The other 40 strains were characterized as CA-MRSA with 80% being *lukPV*-positive. Molecular typing revealed different CA-MRSA clones, most frequently the "Bengal Bay Clone" was found (t345, t657, t5414; ST772). In 39/40 cases CA-MRSA were isolated from SSTIs at different locations; according to this the reason for hospital admission was usually abscess incision and drainage. In 10% of cases the surgical intervention was supported by antibiotic therapy. The median age of the children was 4 years and 36/40 children had a migration background. MRSA-screening (nose and throat) was performed for 21/40 children and 11/40 parents. 52% (11/21) children and 36,4% (4/11) parents were MRSA-positive. Furthermore *S. aureus* was the most isolated species from pediatric abscesses in the study period (173/295 cases) and the majority of strains (70,5%) were MSSA.
Conclusion: Our data highlight the importance of CA-MRSA as a cause of SSTI especially in children. Physicians should be encouraged to increase microbiological and molecular analysis of theses strains to provide more data on CA-MRSA epidemiology in the future.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

668/KMP

Functional characterization of mycobacterial protein PE18/Rv1788 and its ortholog MIP_03868: a comparative analysis

A. Ehtram*[1], Y. Singh[1], S. Kohli[1], S. E. Hasnain[1]
[1]*Indian Institute of Technology Delhi, School of Biological Sciences, Molecular Infection And Functional Biology Lab, New Delhi, India*

Gradual expansion of *Mycobacterium tuberculosis* PE/PPE family of proteins, present exclusively in the genus Mycobacterium, from non-pathogenic to pathogenic mycobacteria and their association with other virulent factors support their role in mycobacterial virulence and pathogenesis. Based on phylogenetic analysis the evolution of PE/PPE family proteins is synchronised with the Esx or Type VII secretion systems from the saprophytic slow growing mycobacteria to the pathogenic fast growing mycobacteria down the geological timescale. The recently evolved Esx5, an exclusive feature of the slow growing pathogenic mycobacteria, serves as the channel for transport of various associated as well as non-associated PE/PPE proteins which lack classical signal peptides. The cell wall association or secretion of PE/PPE proteins contributes emphatically to their multiple roles in mycobacterial virulence including host immune evasion, immune-modulation and antigenic variation. The *esx-5* region of *M.tb* (*rv1782-rv1798*) harbors 2 *pe* (*pe18*, *19*) and 3 *ppe* (*ppe25*, *26*, *27*) genes. The given PE/PPE proteins of this recently evolved secretion system need to be explored which may provide an insight into the molecular basis of mycobacterial infection. A perturbed state is achieved in determining the potent but complex role of these family of proteins because of gene cooption as the orthologs and paralogs may hold significantly distinct role in mycobacterial species other than *M.tb* complex. M-coffee alignment showed a difference of 8 amino acid residues in orthologous proteins PE18/Rv1788 and MIP_03868, respectively. Out of 8 amino acids 3 were found to be conserved whereas 4 amino acids were semi-conserved. Furthermore, phosphorylation sites were detected to be altered between the two homologs as determined by NetPhos software. String analysis suggests interaction of PE18 with PPE25 and PPE26 of Esx5. The localisation of both the proteins was found to be different at sub-cellular level by fluorescence microscopy, where PE18 is found to migrate to mitochondria that is functionally involved in respiration and intermediary metabolism. Contrastingly, its ortholog MIP_03868 was not found to co-localise to the mitochondria. These analyses provide cues to explore the differences in the functionality of these proteins based on the differences in just 8 amino acids or their combinations.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

669/KMP

Species-specific activity of antibacterial drug combinations

A. R. Brochado*[1]
[1]*European Molecular Biology Laboratory, Heidelberg, Germany*

Drug combinations and drug re-purposing can act as a first line of defence against the alarming rise of multi-drug resistant (MDR) bacterial infections, yet their current use in clinics is limited. To better understand the potential of drug combinations, we profiled

~3,000 combinations of different antibiotics, selected human-targeted drugs and food additives in 6 strains from 3 prominent gram-negative pathogens, *E. coli*, *S.* Typhimurium and *P. aeruginosa*, at different drug dose stoichiometries. Although the three species are phylogenetically rather close, >70% of the drug combinations had species-specific effects and >15% of the interactions were even strain-specific, highlighting a vast potential for narrow-spectrum and individualized therapies. Overall, antagonism was twice as common as synergy, but synergies were more conserved across species and enriched in combinations containing non-antibiotics. Moreover, antagonism was almost exclusive between drugs targeting different processes, whereas drugs targeting the same process were heavily biased towards synergism, which has direct mechanistic implications. To illustrate the potential clinical utility of the discovered drug synergies, we demonstrated that 5 of them were effective against clinical MDR *E. coli* or *Klebsiella pneumoniae* isolates.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Food Microbiology and Food Hygiene (FG LM)

670/LMP

Abstract has been withdrawn.

671/LMP
Surveillance of Campylobacter and Arcobacter species from different poultry farms in Thuringia, Germany

E. Zuchantke*[1], H. Hotzel[1], I. Hänel[1], H. Tomaso[1]
[1]*Friedrich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany*

Question: *Campylobacter* spp. have become an increasing concern and are the most common foodborne disease caused by bacteria [1]. The consumption of poultry meat is considered a major source of infection. *Arcobacter* belongs to the family *Campylobacteraceae* and is an emerging pathogen [2]. Therefore the surveillance of poultry farms is an important step to assess the risk and to prevent outbreaks in the future.
Methods: For this purpose, several poultry farms across the federal state Thuringia in Germany were tested in the years 2015 and 2016 by collecting feces samples from geese and ducks. More than 400 samples were investigated using a real-time PCR system to identify *Campylobacter coli*, *Campylobacter jejuni* and *Arcobacter* [3].
Results: A comparison of the two years showed an increase of the number of positive samples for all three bacteria species. A total of 205 samples were tested in 2015 and 240 samples in 2016, respectively. The *Arcobacter*-positive samples increased from 145 samples (71%) in 2015 to 221 (92%) samples in 2016. *C .coli*-positive samples increased from 36 samples (18%) in 2015 to 72 samples (30%) in 2016. *C. jejuni*-positive samples increased from 163 samples (80%) in 2015 to 213 samples (89%) in 2016.
The comparison of the results for ducks shows increasing numbers of *Arcobacter*-positive samples, whereas the number of *Campylobacter* spp. positive samples seems to be stable. The positive findings for geese show an increase for all of the three tested bacteria.
Conclusion: The surveillance of poultry farms is important to determine the risk of foodborne diseases caused by *Campylobacter* and *Arcobacter* species. Screening for antimicrobial resistance is an urgent issue which is currently addressed in new studies.

References
[1] *The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012*. EFSA Journal, 2014. 12(2): p. 3547.
[2] Collado, L. and M.J. Figueras, *Taxonomy, epidemiology, and clinical relevance of the genus Arcobacter.* Clin Microbiol Rev, 2011. 24(1): p. 174-92.
[3] Best, E.L., et al., *Applicability of a rapid duplex real-time PCR assay for speciation of Campylobacter jejuni and Campylobacter colidirectly from culture plates.* FEMS Microbiology Letters, 2003. 229(2): p. 237-241.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

672/LMP
Predictability of ionic liquid toxicity from a SAR study on different systematic levels of pathogenic bacteria

N. Weyhing-Zerrer[1], T. Gundolf[2], R. Kalb[3], R. Oßmer[1], P. Rossmanith[2], P. Mester*[2]
[1]*Merck KGaA, Life Science, Darmstadt, Germany*
[2]*Christian Doppler Laboratory for Monitoring of Microbial Contaminants, Vienna, Austria*
[3]*Proionic GmbH, Graz, Austria*

Introduction: Ionic liquids (ILs), a new class of solvents with unique and tunable physicochemical properties, were initially envisioned as working alternatives to traditional organic solvents. However, they have now proven to have a wide range of alternative chemical and biochemical applications. Due to their increasing use, environmental and toxicity concerns are growing, but resolutions are hindered by the sheer number of possible variants.
Objectives: In order to assess and possibly predict IL-toxicity, a structure activity relationship (SAR) approach was adopted using defined structural motifs. These included varied cationic alkyl side-chain lengths, cation lipophilicity and diverse anion effects.
Materials and Methods: The predictive powers of such SARs in respect of antibacterial effects were compared using a total of 28 ILs on 12 diverse pathogenic bacteria. Endpoints were minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC).
Results: The well recognized side-chain effect was confirmed and imidazolium-based cations compared with commonly used anions. The fluorinated anions had no influence on toxicity with the exception of the [FAP] anion. The effect of this anion was strongly species-dependent and no general trend was observed. Further investigations are required to clarify these significant differences. For all bacteria tested, increased lipophilicity led to increased toxicity. Quaternary ammonium- and phosphonium-based ILs were very toxic for all Gram-positive bacteria tested as well as atypically for Gram-negative *A. fischeri*. These ILs were less toxic for other Gram-negative bacteria and some compounds showed no significant toxic effects. While the most hydrophobic anions, [Docusate] and [NTf_2], can significantly lower cation toxicity, sweeping predictions from few bacteria species and the significance of Gram classification remain challenging issues.
Conclusion: This study provides an overview of the varied toxicities of ILs in clinically-relevant bacteria and cautions against SAR extrapolations from studies conducted with a small diversity of bacteria. Other factors appear to play important roles as evidence by unexpected responses from *A. fischeri*. This strain is used as a model organism, for example, in DIN EN ISO 11348 to monitor the water quality, but its sensitivity to ILs is atypical in respect of other Gram-negative bacteria. Further, the effects of the [FAP] anion and quaternary ammonium- and phosphonium based ILs indicate that IL-induced bacterial toxicity is not well understood. Generalized predictions are yet not possible. Differences in the external structure of these two bacteria phyla may play an important role in IL-sensitivity.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

673/LMP

Phage display for selection of Listeria surface protein specific peptides

J. Bittner*[1], S. A. Funke[1]
[1]*Coburg University, Coburg, Germany*

Listeria monocytogenes is a pathogenic bacterium responsible for foodborne infections and listeriosis, a disease with a high mortality rate. *L. monocytogenes* is able to enter, survive and multiply in different cells [1]. Entry into human epithelial cells requires expression of Internalin A (InlA), a bacterial surface protein, which recognizes and binds E-cadherin, a surface protein of human cells. This interaction induces the internalization into host cells and triggers the infection [2]. In spite of treating with antibiotics, 20 - 30 % of clinical infections result in death [3]. High-risk groups, such as newborns, pregnant women and immunocompromised patients, are especially affected [4]. The current commercially available tests for listeria are mostly culture techniques which are labour and time intensive. To enhance the detection and identification of the pathogen, more rapid methods are needed to meet current demands for food safety testing.
Using a technique called phage display, we develop specific peptides that bind to the surface protein Internalin A of the bacteria. Labeling of these peptides with a fluorophore can facilitate the detection of listeria in human cells or in food samples. Additionally, these selected InlA binding peptides might be used to inhibit the interaction between Internalin A and human E-Cadherin and thus to avoid the entry of the pathogen into host cells.

References

[1] Sheehan B et al, *Curr Top Microbiol Immunol* 192, 187 – 216 (1994).
[2] Menguad et al, *Cell* 84, 923 – 932 (1996).
[3] Wing EJ et al, *J Infect Dis* 185, 18 - 24 (2002).
[4] Amaya-Villar et al, *BMC Infect Dis* 10:324 (2010).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

674/LMP

Enterohemorrhagic *Escherichia coli* O157:H7 strain Sakai can be internalized into the root tissues of corn salad and lettuce following contamination of the soil under greenhouse conditions

K. Eissenberger[1], D. Drissner[2], N. Saile[1], A. Weiss[1], H. Schmidt*[1]
[1]*University of Hohenheim, Institute of Food Science & Biotechnology, Food Microbiology & Hygiene , Suttgart, Germany*
[2]*Agroscope, Microbiology of Plant Foods, Wädenswil, Switzerland*

Introduction: Increasing numbers of cases of human infectious diseases are associated with the consumption of contaminated fresh produce such as sprouts, spinach or salad. These may be contaminated with enterohemorrhagic *Escherichia coli* (EHEC), which have a low infective dose of 10 to 100 cells. The contamination may already occur directly on field, e.g. *via* irrigation water, surface water or manure. Several studies showed EHECs ability to grow on plant leaves and to colonise them. However, it is still unknown if EHEC is able to infect and colonise plants *via* their roots under field conditions.
Objective: In this study we investigated the uptake of *E. coli* O157:H7 into the roots of corn salad and lettuce.
Materials and Methods: Both lettuce cultivars were grown in non-sterile diluvial sand for three weeks. Subsequently the plants were contaminated with fluorescently labelled EHEC O157:H7 strain Sakai *via* irrigation water. After incubating for two to four days at 21°C and 12 h day/night-cycle, plants were excavated and washed followed by disinfection of the root surface. The roots were analysed by fluorescence microscopy for qualitative analysis as well as by homogenisation and spread plating in order to determine the number of internalised bacteria.
Results: Using fluorescence microscopy after disinfecting the root surface, it was shown that under greenhouse conditions *E. coli* cells can be detected within the root tissues. After homogenisation and spread plating of disinfected roots, we were able to detect EHEC and the colony forming units per gram of root (cfu/g) were calculated. The number of cfu/g correlated with the infective dose of EHEC or was even higher. Interestingly, deletion of EHECs adherence related genes *hcpA* or *iha* led to decreased quantities of detected bacteria for both salad cultivars. These results imply that HcpA and Iha play a role during infection of plant roots.
Conclusions: In this *in vivo* study it was shown that under the tested conditions EHEC O157:H7 strain Sakai is indeed able to internalise into root tissues of corn salad and lettuce. The mutants lacking either *hcpA* or *iha* showed reduced ability for internalisation pointing to a role of HcpA and Iha in the colonisation of plant roots. Therefore, further studies are needed to reveal whether these proteins are involved in adherence or in internalisation.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

675/LMP

Selective media for the detection of toxin-producing *Bacillus cereus* and aerobic spoilage bacilli

E. Nischler*[1], G. Kudernatsch[1], M. Ehling-Schulz[1], M. Wagner[1], B. Stessl[1]
[1]*University of Veterinary Medicine Vienna, Department for Farm Animals and Veterinary Public Health, Vienna, Austria*

Bacillus cereus sensu lato (s.l.) is associated with both foodborne intoxications and spoilage of food products. The genetically nearly indistinguishable subdivision includes highly pathogenic, harboring toxin genes (e.g. *ces, nhe, hbl, cytK*), as well as apathogenic species. The aim of this study was to evaluate the limitations and benefits of four chromogenic *Bacillus cereus* agar media in comparison to the ISO recommended standard plating medium by applying a specified strain panel for inclusivity and exclusivity testing. The strain set for inclusivity testing comprised *Bacillus cereus* group strains covering toxin profile A (nhe/hbl/cytK; n=30), C (nhe/hbl; n=37), D (nhe/cytK; n=17), E (nhe/ces; n=6), F (nhe; n=17), G (cytk; n=1) and four further uncharacterized strains (*B. toyonensis*, *B. pseudomycoides* and *B. weihenstephanensis*). Furthermore, aerobic spoilage associated Bacilli (n=45) were included in the test panel (e.g. *B. pumilus*, *B. licheniformis* and *B. subtilis*). The exclusivity strain set include Gram-negative competitors (n=39) and Gram-positive competitors (n=30). The test set was additionally confirmed by biochemical profiling, growth characteristics at 4, 7 and 42°C (in case of *B. weihenstephanensis*), 16S rRNA gene sequencing and conventional PCR methods.The working cultures were grown to early stationary phase and streaked on the test media by a semi-quantitative three loop technique. After incubation for 24 and 48 h at 30 and 37 ± 1 °C, plates were observed for qualitative recovery and colony morphology. Bacterial growth was recorded and the intensity of growth assessed. The *Bacillus cereus* group test strains resulted in a 100 % inclusivity for all media except HiCrome™ agar. The colony morphology was atypical on some media especially for *B. weihenstephanensis*, *B. pseudomycoides*, *B. mycoides* and *B. toyonensis* strains (panC group I, V-VI). The differentiation potential of *B. cereus* group isolates in typical and atypical especially on Hicrome™ agar and Brillance™ agar is a major advantage in food analysis.
Aerobic spoilage associated Bacilli were identifiable due to their characteristic colony morphology. A large variety in the mannitol fermentation and ß-D glucosidase reaction was detectable even

within the same species (e.g. *B. circulans*, *B. licheniformis*). Poor growth conditions for the non-Bacillus cereus group resulted in the highest inhibition rates on CHROMagar™ (n=20) and Bacara® agar (n=25). No growth was only to a certain extend correponding to the higher incubation temperature (37 °C) and seems to be linked to a more effective antibiotic supplement composition.
The results demonstrate the challenging situation in the detection of *Bacillus cereus* group and aerobic spoilage associated *Bacilli*. By characterizing the strain set in a more expansive way the benefits and limits for each application purpose were shown and could support the selection of the best suitable selective agar. Though alternatives as MALDI-TOF MS or PCR methods are available for a rapid confirmation, a higher specificity of conventional *Bacillus* selective media would be mandatory for routine diagnosis.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

676/LMP
Molecular analysis of the impact of *Salmonella* adhesive structures on the adhesion to salad
L. Elpers*[1], M. Hensel[1]
[1]*University of Osnabrueck, Microbiology, Osnabrueck, Germany*

Introduction: *Salmonella enterica* serovar Typhimurium (STM) is a foodborne pathogen which causes gastroenteritis and typhoid fever. The most prominent way of STM to infect humans is by contaminated animal products. However, STM can be spread by vegetables, fruits, herbage and spice, too. This path of infection increases in consequence of healthier lifestyles and the availability of prepacked salads and vegetables. The consumption of these products in a raw manner increases the risk of infections. This fact is also shown by hundreds of *Salmonella* outbreaks around the world caused by fresh products.
The mechanisms how bacteria attach to different salads and vegetables and invade into plant tissues are still unknown. In this context the large repertoire of adhesive structures which involve fimbrial adhesins, non-fimbrial adhesins as well as outer membrane proteins (OMPs) and flagella are taken into account. As only a few of these adhesive structures are expressed under laboratory conditions and in mammalian hosts, we hypothesize their relevance under different environmental conditions, thereby contributing to the colonization of various non-host environments.
Objectives: The aim of this project is the establishment of an experimental system to investigate and analyze the impact of various adhesive structures of STM on the adhesion to fresh products, e.g. corn salad, lettuce and spinach.
Material & Methods: To establish an assay to analyze the impact of adhesive structures on fresh products various parameters were tested, including duration of infection, washing steps, type of homogenization, buffers and the amount of bacteria. For the adhesion assay corn salad (*Valerianella locusta*) was seeded and grown under sterile conditions for 8 weeks at 20 °C with a 12 h light/12 h dark rhythm. Quantitative adhesion assays were performed using strains with Tet-inducible expression of adhesins from *Salmonella enterica*.
Results: We established an assay to quantify the role of each of the various adhesins of STM in adhesion to fresh products. The adhesion assay with corn salad is applicable to other fresh products e.g. lettuce (*Lactuca sativa*). Adhesins of EHEC can also be expressed by *E. coli* laboratory strains to investigate their role in adhesion to fresh products.
Conclusion: Testing the impact of all adhesive structures of STM by the newly established adhesion assay with various kinds of salad will provide insights into the contamination process of STM among fresh products. Using the experimental system to analyze the impact of adhesive structures of in general food-borne pathogens will help to prevent large foodborne outbreaks caused by consumption of fresh products.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

677/LMP
Functional analysis of TLR5 receptors from different species
E. Faber*[1], S. Kunze[1], K. Tedin[2], Y. Speidel[1], D. Hlahla[1], C. Josenhans[1]
[1]*Hanover Medical School, Medical Microbiology and Hospital Epidemiology, Hanover, Germany*
[2]*Free University Berlin, Microbiology and Epizootics, Berlin, Germany*

Question: Toll-like receptors (TLRs) are conserved innate immune receptors, which play a crucial role at first line of defense during host-pathogen interactions. By recognition of conserved microbial structures TLRs are also contributing to the maintenance of immune homeostasis. Bacterial flagellin is a strong trigger of Toll-like receptor 5. The importance of TLR5 signaling in evolution becomes obvious by the strong conservation of the receptor among vertebrates: from fish to mammals. With the aid of chimeric TLR5 receptors, we compared TLR5 signaling of different species (human, chicken, mouse, pig, cattle) within one common cellular system.
Methods and Results: Chimeric TLR5 receptors, consisting of extracellular domains from different animal species (chicken, mouse, pig, cattle) linked to human intracellular domains, were successfully generated and transiently expressed in different cell lines. Activation assays using purified *Salmonella* flagellin confirmed functionality of all designed chimeric receptors within human cells, marked by elevated NF-κB activation, IL-8 secretion and posttranslational modification of downstream signalling molecules. Moreover, comparison of activation profiles of chimeric TLR5s and their animal counterparts revealed significant differences regarding functionality within different human cellular backgrounds. Exploitation of chimeric receptors as a tool allows for the identification of activation potential exclusively dependent on receptor ectodomains. We will present results of testing for species-specific differences using purified *Salmonella* flagellin and *Salmonella* and *Campylobacter jejuni* lysates.
Conclusions: We conclude, that several intracellular determinants of TLR5 are crucial for compatibility with the species background and therefore for the proper functionality of the receptor within a heterologous expression system. By using chimeric TLR5 receptors with a common intracellular domain we provided an expression system to tackle bacteria-specific differences in receptor activation.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

678/LMP
Microbial safety of ethnic foods
B. Stessl*[1], E. Steinkogler[2], M. Heindl[2], S. Muri-Klinger[1], K. Brugger[1], M. Wagner[1]
[1]*University of Veterinary Medicine Vienna, Department for Farm Animals and Veterinary Public Health, Vienna, Austria*
[2]*University of Vienna, Vienna, Austria*

The consumption and demand of ethnic food and food imported from remote countries is growing, most likely due to the globalization of food supply chains and cosmopolitan eating habits.
Recently, the EU funded research project PROMISE has shown that consumer exposure to zoonotic bacterial agents (Salmonella spp., Shiga-toxin producing Escherichia coli (STEC), Listeria monocytogenes and Staphylococcus aureus) transmitted by illegally imported food vehicles is not neglectable. Ready-to-eat food or their ingredients (spices, sprouts, traditional cheeses, fish, seafood and meat) are most often involved in larger foodborne

outbreaks. Failure to detect or estimate bacterial pathogens can cause adverse health effects and fatalities besides substantial economic losses.
For the first time we focus on the food safety of ethnic food sold in specialty shops and local food markets in the multi-cultural city Vienna. In the baseline approach ethnic food in the category fish and fish products, meat and meat products, vegetables, sprouts, cheeses and spices will be taken at retail level, following a tailor-made sampling concept. Data concerning category, import routes, origin, packaging, physico-chemical parameters and microbiological status will be collected. Pathogenic bacterial isolates (e.g. L. monocytogenes, Staphylococcus aureus) will be characterized by antibiotic susceptibility testing and the combination of gold standard molecular epidemiological tools [multi-locus sequence typing (MLST), spa-typing] and whole genome sequence typing (WGS). This approach should help to gain more insight into the global spread of certain genotypes via ethnic food and their virulence potential. The outcome of this study should support consumers, food business operators, retailers and food authorities to estimate the risk of foodborne pathogens present in ethnic food.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

679/LMP

Investigation of the potential of dry ice blasting for cleaning and disinfection in the food production environment

A. K. Witte*[1], M. Bobal[1], R. David[2], B. Blättler[3], D. Schoder[1], P. Rossmanith[1]
[1]*Institut für Milchhygiene, Vienna, Austria*
[2]*Minus80Grad, Perchtoldsdorf, Austria*
[3]*LEU Anlagenbau AG, Uetendorf, Switzerland*

Introduction: For dry ice blasting solid CO2 (-78°C) pellets are applied at high pressure to the surface where it immediately sublimates. It is environmentally friendly, fast and residue-free and thus offers many advantages. Although often applied in many industry sectors, little information is available with respect to the disinfection potential of dry ice blasting. Only a few studies showed that dry ice blasting can reduce bacterial counts on different surfaces in food processing areas.
Objectives: The aim of the study was to evaluate whether dry ice blasting can be used as disinfection method and to clarify which factors influence the disinfection/cleaning potential. Furthermore, the study examined the risk of recontamination.
Materials & methods: For the investigation of dry ice blasting under standardized conditions, a fixed device constructed by our laboratory was used. We selected the ubiquitous Gram-positive bacterium *Micrococcus roseus* as surrogate because it is non-pathogenic and its intensive red color is easily detected. Detection of the bacterium was realized with contact plates. The parameters (i) quantity of CO2 pellets, (ii) pellet size, (iii) pressure, (iv) influence of the initial amount of contaminating bacteria, and (v) recontamination caused by aerosols were investigated. Since the study focused on cleaning food processing areas, different materials were compared including standardized tiles and materials derived from smear robots.
Results: Dry ice blasting removes bacterial cells to a similar extent from several surfaces and components of dairy production equipment, occasionally with a slight abrasive effect. Efficacy is affected by the quantity of dry ice and pressure applied but neither by the pellet size nor the initial quantity of bacterial cells on the surface. Since the bacteria removal rate is less than five log10 units, dry ice blasting cannot be recommended as a disinfection method, but it demonstrates efficient cleaning comparable to other conventional methods. Viable cells were frequently detected in the surrounding area after dry ice blasting, showing that there is a high risk for contamination.
Conclusion: Due to the missing disinfection potential of dry ice blasting, it is only recommended as cleaning method with a subsequent disinfection step. In practice, dry ice blasting of food production equipment should take place outside the production area because there is a high risk of recontamination due to spread of the bacteria.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

680/LMP

Sensitive detection of Ricin in complex matrices

H. von Buttlar*[1], P. Kriebs[1], P. Vollmar[1]
[1]*Bundeswehr Institute of Microbiology, Bacteriology and Toxinology, München, Germany*

Introduction: Ricin, the toxic ingredient of *Ricinus communis* seeds, is a protein composed of an A and a B chain linked by a disulphide bridge. While the B chain facilitates binding to target cells by its lectin moiety, the A chain acts as ribosome inactivating protein. Ricin might potentially be abused as a biowarfare or bioterroristic agent. To assault larger groups, besides aerosolisation, food chain contamination with the toxin is a potential scenario. Therefore, the detection of Ricin in complex food matrices is of great importance.
Objectives: An assay for the sensitive detection of Ricin in complex matrices should be established. The analysis of food matrices often requires pre-analytical preparations. Here, the toxin is diluted so the analysis of a bigger sample volume is beneficial to reach high sensitivity.
Materials and methods: A protocol was established to enrich Ricin with paramagnetic microspheres coated with Ricin-specific antibodies from PBS. Ricin bound to the beads was then detected by enzyme conjugated antibodies. After substrate reaction the supernatant was transferred without beads and Optical Density (O.D.) was measured. Sensitivity and specificity was determined with crude extracts of castor seeds and highly purified Ricin. Afterwards food matrices were spiked with Ricin and analysed with the new established On-Bead ELISA
Results: An ELISA was established to detect Ricin in complex matrices based on immuno-magnetic enrichment. Enzyme linked detection is done directly on the beads. So the sample volume processed is not limited as it is in classical ELISA. Nevertheless, the assay is compatible with standard laboratory equipment. The system is able to detect Ricin in various food matrices, including dairy products.
Conclusion: Ricin can highly sensitive be detected in complex matrices by this On-Bead ELISA. Moreover, the assay can be conducted with standard laboratory equipment enabling wide distribution of the assay.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

681/LMP

Has been changed into 035/LMV.

682/LMP

Antifungal acids produced by lactic acid bacteria

L. Lipinska*[1], M. Sojka[2], R. Klewicki[2], R. Bonikowski[3], K. Kolodziejczyk[2], E. Klewicka[1]
[1]*Politechnika Lodzka (Lodz University of Technology), Institute of Fermentation Technology and Microbiology, Lodz, Poland*
[2]*Politechnika Lodzka (Lodz University of Technology), Institute of Food Technology and Analysis, Lodz, Poland*
[3]*Politechnika Lodzka (Lodz University of Technology), Institute of General Food Chemistry, Lodz, Poland*

Introduction: Lactic acid bacteria (LAB) can produce a variety of specific antifungal agents such as ethanol, the cyclic dipeptides or the low-molecular-weight proteins. From those, the acidic metabolites of LAB play the most crucial role in inhibition of fungi (molds and yeasts) and can be used to control the microorganisms causing food spoilage.
Objectives: The aim of our study was to analyze and to determine the antifungal acidic compounds in the supernatants after lactic acid fermentation of selected strains *Lactobacillus* spp. bacteria.
Materials & methods: We characterized and quantified the most relevant antifungal acids after 48-hour lactic acid fermentation of five strains of *Lactobacillus* spp. bacteria. We selected bacteria from a group of sixty strains, focusing on antagonistic activity against yeasts (two strains of *Candida* sp.), and molds (*Alternaria alternata, Alternaria brassicicola, Aspergillus niger, Aspergillus ochraceus, Fusarium latenicum, Geotrichum candidum, Mucor hiemalis, Penicillium* spp.). Lactobacilli with the most significant antifungal properties were chosen and cultivated in MRS broth medium (Merck) under optimal conditions (30°C ÷ 37°C). The cell-free supernatants were obtained by centrifuging and the filtration of the inoculated and fermented medium. The samples were stored at -20°C.
We used the enzymatic method to determine DL-lactic-, and acetic acid concentration, and HPLC-MS method combined with Quechers technique for other acids. Additionally, we examined the occurrence of hydroxy fatty acids in the cell-free supernatants by GS method.
Results: We have identified and quantified the antifungal acids after lactic acid fermentation of chosen strains. Lactobacilli produced a substantial amount of DL-lactic acid (10.7 ÷ 16.7 $g \times L^{-1}$), particularly L-lactic acid, acetic acid (4.0 ÷ 5.7 $g \times L^{-1}$), and DL-3-phenyllactic acid (39.6 ÷ 72.4 $mg \times L^{-1}$). The amount of DL-p-hydroxyphenyllactic acid (4.3 ÷ 9.7 $mg \times L^{-1}$), and benzoic acid (0.5 ÷ 0.8 $mg \times L^{-1}$) was also significant. The content of the other low-concentrate (<0.5 $mg \times L^{-1}$) acidic antifungal agents was determined as: hydrocaffeic acid, hydrocinnamic acid, vanillic acid, 4-hydroxybenzoic acid, catechol, caffeic acid, ferulic acid, 3-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid, p-coumaric acid.
Several fatty acids were detected including hydroxy fatty acids, such as 2-hydroxy-4-methylpentanoic acid, the compound well-known as a strong antifungal agent.
Conclusion: The examined strains of lactobacilli produced antifungal acids both as a primary (lactic acid, acetic acid) and secondary metabolites. Chemical structures and concentrations of analyzed agents depend on bacterial strain.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

683/LMP

***Lactobacillus* sp. – efficient producers of exopolysaccharides**

M. Oleksy*[1], E. Klewicka[1]
[1]*Lodz University of Technology, Institute of Fermentation Technology and Microbiology, Lodz, Poland*

Lactobacillus sp. have the ability to produce exopolysaccharides (EPSs) which play economically important role in the production of fermented foods, improving the gustatory and rheological properties of the finished products. These polymers can be treated as natural biothickening agents because they are produced *in situ* by the *Lactobacillus*-starters that have General Recognized As Safe status (GRAS). Due to GRAS classification *Lactobacillus* sp. are likely candidates for the production of functional EPSs on global scale. Everything also indicates to the fact that, EPS from *Lactobacillus* sp. has health benefit properties like antioxidant, anti-ulceretic, anti-tumor and immunomodulating activities. Although the EPS from *Lactobacillus* sp. offers many opportunities as thickening agents their production is unprofitable because of the low yield of the synthesis. Optimization of culture conditions can be a first step towards enhancing biosynthesis EPSs by *Lactobacillus* sp.1,2)
The aim of this work is to optimize the production of exopolysaccharides by a probiotic *Lb. rhamnosus* ŁOCK 0943, ŁOCK 0935 and the strain designated as OM-1 in order to maximize production of EPS. Tested strains was obtained from the collection ŁOCK 105, Lodz, Poland.
The influence of the following sources of carbon on the production of EPS was tested in one-factor- and multi-factor-at-a-time experiments: glucose, galactose, fructose, lactose, sucrose, and maltose at a concentration of 20 g/l in MRS medium. EPS production was determined by measuring carbohydrate concentrations using colorimetric methods.3)
Plackett-Burman design was used for optimization of culture conditions for exopolysaccharides production. It was studied effect on seven variables at two levels - a high level denoted by (1) and a low level denoted by (−1). All experiments were conducted in triplicate, and the average value of EPS yield was used for statistical analysis.
This study showed that culture conditions have a clear impact on EPSs production by *Lactobacillus* sp. The most important factors affecting the level of expolysaccharides production are carbon sources - saccharides. In turn protein components are insignificant and might not create high impact if their level in the culture medium is changed.

References

[1] Freitas F., Alves V.D., Reis M.M.A. (2011). Advances in bacterial exopolysaccharides: from production to biotechnological applications. Trends Biotechnol. 29 (8): 388 – 396.
[2] Polak-Berecka M., Waśko A., Kubik-Komar A. (2014). Optimization of Culture Conditions for Exopolysaccharide Production by a Probiotic Strain of Lactobacillus rhamnosus E/N. Pol. J Microbiol. 63 (2): 253 – 257.
[3] Dubois M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. (1956). Colorimetric method for the determination of sugars and related substances. Anal. Chem. 28: 350–356.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

684/LMP
Proteolytic activity of *Pseudomonas* spp. isolated from raw milk
G. Lücking*[1], M. von Neubeck[1], C. Huptas[1], S. Scherer[1], M. Wenning[1]
[1]*Technische Universität München, Lehrstuhl für Mikrobielle Ökologie, Freising, Germany*

Spoilage of UHT milk and milk products is often caused by heat-resistant peptidases produced by psychrotolerant microorganisms during raw milk storage. Our previous study on the microbiota of raw milk resulted in *Pseudomonas* being the most abundant genus as well as the genus with the highest proteolytic activity [1]. Although over 30 different *Pseudomonas* species were identified in raw milk, 60% of the isolates could be assigned to four main species, namely *P. proteolytica, P. lundensis, P. fragi* and one novel hitherto unknown *Pseudomonas* species. Screening of the enzymatic activity by agar diffusion assays showed great variations among the isolates. So far, only one extracellular metallopeptidase (AprA) is known in *Pseudomonas*, which is encoded by a gene of the *aprA-lipA* operon. In *P. fluorescens* this operon was characterised as a gene cluster consisting of eight genes, which code for a peptidase, an inhibitor, a type I secretion system, two putative autotransporter and a lipase.
In this study the organization and regulation of the *aprA-lipA* operon in *Pseudomonas* species, which are predominant in milk, was analysed. For this, whole genomes of 60 milk isolates (belonging to eight different *Pseudomonas* species) were determined by NGS. Sequence analysis revealed species- and strain-dependent differences in the presence and localisation of *aprA-lipA* operon genes. Furthermore, the proteolytic activity of the milk isolates was quantified by an enzyme assay using azocasein as substrate. The activity of AprA varied strongly in a strain-specific manner and was also shown to be influenced by external factors like incubation temperature and media. A correlation between certain peptidase phenotypes and *aprA* operon structures was observed to some extent; however, peptidase production in *Pseudomonas* spp. seems to underlie more complex regulation mechanisms.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Microbial Cell Biology and Cellular Microbiology (FG MCB)

685/MCBP
Chromosome organization in *Corynebacterium glutamicum*
K. Böhm*[1], M. Bramkamp[1]
[1]*Ludwig-Maximilians-Universität München, Fakultät Biologie, Planegg-Martinsried, Germany*

Bacterial chromosome replication goes hand in hand with DNA segregation and condensation, which are inevitable processes for cell growth and division. Separation of sister chromosomes can be mediated by the ParABS system. Thereby, the ParB protein binds centromere-like *parS* sequences in the proximity of the origin of replication (*oriC*) and interacts with the DNA-binding ATPase ParA mediating translocation of sister chromosomes towards the opposite cell halves. Besides this, SMC/ScpAB complexes are implicated in DNA segregation by entrapping and condensing the nucleoid. These major mechanisms coordinating chromosome organization have so far been uncharacterized in *Corynebacterium glutamicum*, a rod shaped, polar growing actinobacterium widely used for industrial production of metabolites like amino acids and vitamins. Therefore, we aim to elucidate the spatiotemporal chromosome segregation via Par proteins, the function of the *C. glutamicum* condensin complexes SMC/ScpAB and MksBEF (MukBEF-like SMC) as well as interaction between both systems.
Spatiotemporal localization patterns of the ParABS system were studied using allelic replacements of Par proteins by fluorescently tagged versions. Analysis was performed by fluorescence microscopy and on a single cell level in microfluidic chambers. Further, ChIP analysis served to identify *parS* sites *in vivo*. In order to investigate the impact of condensins on chromosome organization deletion mutants were characterized, while subcellular localization of fluorescent SMC protein was studied microscopically. Putative interactions of condensins and Par complexes were analyzed using bacterial-two-hybrid assays.
Here we show that *C. glutamicum* harbors 10 *parS* sites located close to *oriC*, which recruit ParB protein. ParA is stably enriched as bright foci at both cell poles and dynamically localizes to septal sides in a late stage of the cell cycle. Coexpression of fluorescently labeled ParA and ParB revealed colocalization of ParA and ParB-*oriC* nucleoprotein complexes. Both *C. glutamicum* condensin proteins SMC and MksB are dispensable for DNA segregation, *oriC* localization and cell growth. Moreover, fluorescently tagged SMC localizes in multiple distinct foci within the cell, similar to ParB. Finally, our data suggest interaction between the two chromosome organizing systems and support predicted complex formations of SMC/ScpAB and MksBEF.
In conclusion, our results give further insights into the coordination of *C. glutamicum* DNA segregation throughout the cell cycle via the ParABS system, suggest a crosstalk between the three protein complexes ParAB, SMC/ScpAB and MksBEF and, therefore, hint to a putative role of condensins in related processes.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

686/MCBP
Genome-wide screen for the identification of novel factors required for the production and glycosylation of lipoteichoic acid in *Listeria monocytogenes*
J. Rismondo*[1], M. G. Percy[1], A. Gründling[1]
[1]*Imperial College London, London, United Kingdom*

Listeria monocytogenes is an important human pathogen, which can cause severe infections leading to septicaemia, meningitis or encephalitis. *L. monocytogenes* has a typical Gram-positive cell wall, which is composed of peptidoglycan and teichoic acids. Teichoic acids, which include wall teichoic acid (WTA) and lipoteichoic acid (LTA), play an important role for cell viability, cell division and virulence, making them suitable targets for the development of new antimicrobials. Both, LTA and WTA, are modified with D-alanine and sugar residues. While the alanylation process has been intensively studied, the proteins for the glycosylation of LTA are either unknown or not well characterized. Indeed, the first enzyme required for this process, the cytoplasmic glycosyltransferase GtlA has only very recently been identified. The aims of this study are to identify the missing enzymes required for the glycosylation of LTA as well as other novel factors involved in LTA production. For this purpose, a mariner-based transposon system was used to generate a bank of *L. monocytogenes* mutants. Next, these transposon library strains were screened for altered LTA production using an ELISA-based assay and a polyglycerolphosphate specific LTA antibody. The read out of this genetic screen is based on the observation that an *L. monocytogenes gtlA* mutant strain, which lacks galactose residues, yields a stronger signal using this assay. Of the 1,500 transposon mutants screened, six showed a stronger signal in the ELISA-based LTA detection assays. The increase in LTA signal was subsequently confirmed for all six strains by western-blot analysis and the transposon mutant insertion site mapped by arbitrarily primed PCR and sequencing. Four mutants contained an insertion in *lafC*, which has previously been associated with the

LTA biosynthesis process. Deletion of *lafC* results in the production of LTA with an altered structure as assessed by western blot, but the exact function of the encoded protein remains unknown. The other two mutants had transposon insertions in genes coding for the flagellar hook protein FlgE and the penicillin binding protein PBP A2. In summary, using the transposon mutagenesis and ELISA-based LTA detection screen, we were able to identify proteins, which have a direct or indirect impact on LTA biosynthesis and/or LTA glycosylation in *L. monocytogenes*. Further experiments are currently underway to understand the exact role of these proteins in the LTA biosynthesis process. In addition, a larger transposon mutant collection will be screened to uncover additional novel factors. All together this work will help us to gain further insight into the LTA biosynthesis pathway and how it is interlinked with other cellular processes.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

687/MCBP

***Staphylococcus aureus* decreases pro- inflammation of immune cells via wall teichoic acid**

X. Li*[1], R. Kristin[1], K. Petra[1], A. Peschel[1]
[1]*Institut für Infektionsbiologie, Tübingen, Germany*

Staphylococcus aureus is a pathogen whose combat in human health care is becoming more and more important. Wall teichoic acids (WTA) served as receptor for antibody and MBL binding, and its glycosylation enables adherence to nasal epithelial cells in hosts [1]. We investigated the effects of *S. aureus* USA 300 Δ*tagO* mutant strains devoid of WTA, or double mutant *ΔtarMΔtarS* strains, deficient in both α- and β-GlcNAc residues on activation of human dendritic cells (DCs) and macrophages. The mutant strains without glycosylation or WTA induced higher IL-1β and TNF-α release than the wild type in macrophages. Furthermore, we found that both *S. aureus* Δ*tagO* and *ΔMΔS* mutants induced dramatic high IL-8 release in TLR2-transfected HEK293 cells. In western blot analysis we found out that WTA mutant strain expresses and releases more lipoproteins than other strains. Thus we offered propose the hypothesis that strains lacking WTA have a less dense cell envelope and a leaky cell wall. Thus lipoproteins could be released into the extracellular space and activate immune responses. Our findings could offer us a new function of *S. aureus* wall teichoic acid in protecting the bacteria from recognition by antigen presenting cells, thus it could play a potential role in immune evasion.

References

[1] Winstel, V., et al., *Wall Teichoic Acid Glycosylation Governs Staphylococcus aureus Nasal Colonization.* MBio, 2015. 6(4): p. e00632

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

688/MCBP

***Streptococcus pneumoniae* binds Ultra Large Von Willebrand Factor**

J. Hilger*[1], R. Manfred[2], B. Ina-Kristin [1], F. Marcus[3], S. Michael[1], B. Simone[1]
[1]*Institut für Mikrobiologie, AG Steinert, Braunschweig, Germany*
[2]*Helmholtz Centre for Infection Research, Central Facility for Microscopy, Braunschweig, Germany*
[3]*Freie Universität Berlin, Centre of Infection Medicine, Berlin, Germany*

Introduction: As member of the nasopharyngeal microflora, *Streptococcus pneumoniae* (the pneumococcus) colonizes the upper airways. Pneumococci also emerge as causative agent for various local infections, and also for live threatening diseases such as community acquired lobar pneumonia, purulent meningitis, and septicaemia. Vascular endothelial cells serve as important defense barrier against bacterial pathogens circulating in the bloodstream. The immediate anti-inflammatory response is mediated by exocytosis of Weibel Palade bodies thereby releasing cytokines, vasoactive substances and procoagulatory von Willebrand Factor (vWF) into the vessel lumen. Former own cell culture infection studies demonstrated the induction of vWF release from primary endothelial lung cells incubated with *S. pneumoniae* (Lüttge *et al*., 2012)
Objectives: The main topic of this project aims to analyse the interaction between the pathogen *Streptococcus pneumoniae* and human vWF. VWF is circulating in its globular conformation within the blood and also forms long ultra large, aggregated fibers on activated endothelial surfaces exposed to strong shear stress. The role of vWF as endothelial attachment site for pneumococci is analysed in different experimental binding studies.
Materials and Methods: In addition to binding analyses with iodinated vWF protein and different pneumococcus serotypes, the impact of pneumococcal encapsulation on vWF binding was analysed by flow cytometry. Furthermore a microfluidic system (Ibidi®) was established to elucidate vWF binding to a differenciated endothelial cell layer in various flow conditions resembling the blood stream.
Results: The present comprehensive analyses indicate binding of pneumococcus to both, globular vWF proteins circulating in human vasculature and to multimerized, ultra large vWF fibers, generated on the endothelial cell surface. Iodinated vWF protein demonstrated variations in binding activity to various pneumococcus serotypes. Furthermore, flow cytometry analyses revealed a strong dependence of vWF binding on amount of capsule polysaccharides. A strong encapsulation significantly reduces vWF binding. The binding of vWF to floating pneumococci as well as by cell surface-adhered pneumococci is repeatedly detected and quantified by immuno fluorescence microscopy with specific antibody detection. In order to mimic the mechanosensitive conformational change from a globular vWF into ultra large vWF-fibers, binding to pneumococci was analysed using primary human endothelial cells in an Ibidi® microfluidic system. Using this system, bacterial attachment to long vWF fibers for more than 20 min was observed.
Conclusion: The presented vWF-binding data demonstrate that pneumococci recruite vWF for attachment to blood vessel walls even in the bloodstream at high shear rates.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

689/MCBP

A DivIVA-associated SOS response pathway in *Corynebacterium glutamicum*

G. Giacomelli*[1], M. Bramkamp[1]
[1]*LMU, Department 1, Planegg-Martinsried, Germany*

Despite their simple morphology bacterial cells are able to spatially organize different functions with exquisite precision. In *Corynebacterium glutamicum*, the most evident example is the DivIVA-dependent polar localization of the elongasome that results in polar cell growth.
DivIVA is a conserved protein found in many bacteria. DivIVA is able to autonomously recognize regions of high membrane curvature such as cell poles and newly forming septa. At these sites DivIVA forms a polymer structure that acts as a scaffold for a multitude of processes such as chromosome segregation, through the tethering of the ParABS system, and polar positioning of the cell elongation machinery.
DivIVA sequences in members of the actinobacteria are particularly long and we could previously show that distinct regions in DivIVA are responsible for specific interaction

contacts. Here, we set out to identify putative novel DivIVA associated processes. In order to do so, pull-down assays with a Strep-tagged DivIVA were performed and co-eluting proteins were identified via mass spectrometry.
This procedure allowed for the identification of a new interaction partner, DipC (DNA damage induced polar protein C), belonging to a conserved operon found in most actinobacteria. The operon is located downstream a LexA box (SOS response), suggesting an involvement in DNA repair mechanisms. To monitor DipC expression the gene was replaced by a fusion construct encoding a fluorescently tagged version of the protein. SOS response was induced with mitomycin C and the DipC expression was followed over time microscopically. Importantly, DipC localizes to the cell poles and septa were it co-localizes with DivIVA. A *dipC* knockout strain showed decreased cell viability in presence of mitomycin C compared to the wild type *C. glutamicum* strain. We directly visualized an increased number of cells with DNA damage using a TUNEL assays (Terminal deoxynucleotidyl transferase dUTP nick end labelling). Further studies are ongoing to understand the molecular function of DipC and the other proteins that comprise the operon in DNA repair. We also address the necessity and consequences of the spatial confinement of these SOS proteins to the cell poles.
This study identifies and characterizes a novel DivIVA-dependent pathway involved in the SOS response in *C. glutamicum*. Our data point to a spatially confined novel DNA repair mechanism that is associated with apically growing bacteria.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

690/MCBP

Morphogenesis of the stalked budding bacterium *Hyphomonas neptunium*

S. Rosskopf*[1], E. Cserti[1], M. Thanbichler[1]
[1]*Philipps-Universität Marburg, Microbiology, Marburg, Germany*

The spatial and temporal regulation of peptidoglycan biosynthesis and its role in cell morphology has been studied intensively in well-characterized model organisms such as *Escherichia coli*, *Bacillus subtilis*, and *Caulobacter crescentus*, which divide either by symmetric or asymmetric binary fission. To broaden our knowledge of the mechanisms governing bacterial morphogenesis, we have started to investigate the dimorphic marine alphaproteobacterium *Hyphomonas neptunium* as a new model organism.[1] This Gram-negative species is characterized by a unique mode of proliferation whereby new offspring is generated by the formation of buds at the tip of a stalk that emanates from the mother cell body.
The main focus of our studies was the identification of cell wall biosynthetic enzymes and regulatory factors enzymes that are critically involved in stalk and bud biogenesis.[2] To this end, we comprehensively analyzed the localization patterns of all the proteins with cell wall-related functions encoded in the *H. neptunium* genome. Moreover, we probed the essentiality of these candidate proteins by deletion analysis and determined the phenotypes of the resulting strains. These studies revealed that peptidoglycan biosynthesis in *H. neptunium* is a complex process mediated by an intricate interplay of various factors. Using a fluorophore-conjugated D-amino acid to visualize nascent peptidoglycan, we found that the stalk elongates through localized insertion of peptidoglycan at the new cell pole. Later in the cell cycle, growth is largely limited to the nascent bud cell compartment. Consistent with this finding, several biosynthetic proteins localize to these subcellular locations in a cell cycle-dependent manner. Furthermore, we could show that peptidoglycan hydrolases play a prominent role in cell division, even though they are highly redundant. Most likely not all have a function in active cleavage but are involved in the regulation of other proteins. These results for the first time provide insight into the mechanisms of morphogenesis in stalked budding bacteria, thus setting the stage for an in-depth analysis of the regulatory mechanisms that control the spatiotemporal dynamics of the peptidoglycan biosynthetic machinery in these organisms.

References

[1] Jung A, Eisheuer S, Cserti E, Leicht O, Strobel W, Möll A, Schlimpert S, Kühn J, Thanbichler M (2014) Molecular toolbox for genetic manipulation of the stalked budding bacterium *Hyphomonas neptunium*. Appl Environ Microbiol 81, 736-744.
[2] Cserti E, Rosskopf S, Chang Y, Eisheuer S, Selter L, Shi J, Regh C, Koert U, Jensen GJ, Thanbichler M. Dynamics of the peptidoglycan biosynthetic machinery in the stalked budding bacterium *Hyphomonas neptunium*. (under revision)

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

691/MCBP

Control of PgdA-dependent peptidoglycan N-deacetylation by GpsB and PBP A1 in *Listeria monocytogenes*

J. Rismondo[1,2], C. Aldridge[3], W. Vollmer[3], S. Halbedel*[1]
[1]*Robert Koch Institute, FG11-Division of Enteropathogenic bacteria and Legionella, Wernigerode, Germany*
[2]*Imperial College London, Section of Microbiology, London, United Kingdom*
[3]*Newcastle University, The Centre for Bacterial Cell Biology, Newcastle upon Tyne, United Kingdom*

GpsB is a late cell division protein, similar in sequence and structure to DivIVA and strongly conserved in all firmicutes. In the bacteria studied so far, GpsB controls localization and/or function of penicillin binding proteins that contribute to peptidoglycan biosynthesis at the site of cell division (1-3). We are studying GpsB in the human pathogen *Listeria monocytogenes* where GpsB is required for the correct activity of the major bi-functional penicillin binding protein PBP A1 (2,4). We discovered that GpsB influences the resistance of *L. monocytogenes* against lysozyme, an important effector of the innate immune system. Mutant strains lacking *gpsB* were prone to spontaneous autolysis but showed an increased lysozyme resistance. This increased lysozyme resistance could be corrected by deletion of *pbpA1*, demonstrating that PBP A1 is likewise involved in control of lysozyme resistance. Susceptibility to lysozyme mainly depends on two enzymes in *L. monocytogenes*: (i) The peptidoglycan N-deacetylase PgdA and (ii) the peptidoglycan O-acetyltransferase OatA. The increased lysozyme resistance of the Δ*gpsB* mutant coincided with increased N-deacetylation rates detected in peptidoglycan preparations of *gpsB* mutant strains. Moreover, the increased lysozyme resistance of the Δ*gpsB* mutant clearly required PgdA and was independent of OatA, strongly indicating that GpsB negatively controls activity of PgdA. Protein-protein interaction studies support the idea that GpsB, PBP A1 and PgdA form a tripartite complex in *L. monocytogenes* and identified the regions in PBP A1 and PgdA required for complex formation. These and further results establish a physiological connection between GpsB, PBP A1 and the peptidoglycan modifying enzyme PgdA. To our knowledge, this is the first reported link between a DivIVA-like cell division protein and factors important for escape from the host immune system.

References

[1] Claessen *et al.* 2008. *Mol. Microbiol.* 68:1029-1046
[2] Rismondo *et al.* 2016. *Mol. Microbiol.* 99:978-998.
[3] Land *et al.* 2013. *Mol. Microbiol.* 90:939-955.
[4] Rismondo *et al.* 2015. *Mol. Microbiol.* 95:332-351

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

692/MCBP
The Architecture of Chromosome Maintenance Motifs on Bacterial Chromosomes
T. Sperlea*[1], P. Sobetzko[1], T. Waldminghaus[1]
[1]*Philipps-Universität Marburg, LOEWE-Zentrum für Synthetische Mikrobiologie, Marburg, Germany*

As we are approaching the point where assembly and design of complete bacterial chromosomes becomes feasible, we must start to think about the chromosome as more than a simple array of genes. In order for genetic information not to get lost or be corrupted, chromosome maintenance systems are needed. Many of these systems consist of a protein that specifically binds to or acts upon a short DNA motif. The distribution of these motifs on natural chromosomes is nonrandom and proposed to be linked to the function of the respective maintenance system. A systematic study of such chromosome-wide motif patterns is lacking in part because of difficulties in statistical assessments. These arise due to protein coding sequences covering around 90% of bacterial genomes and serving as predominant layer of genomic information.
In order to arrive at a comprehensive set of positioning rules for chromosome maintenance motifs, we used DistAMo, a tool that statistically assesses motif abundance based on codon occurrences [1]. In order to understand general rules for motif distributions, we analyzed chromosomes of 767 bacteria from all major clades. Distribution patterns were investigated for motifs involved in processes such as nucleoid occlusion (SlmA binding site, Noc binding site), sister chromosome separation (KOPS), chromosome condensation (matS), mutation repair (GATC) and recombination (Chi). Our study confirms some previous analyses on individual chromosomes but additionaly reveals new conserved distribution patterns in great detail. The results raise new questions on the modes of function of the investigated maintenance systems. Furthermore, in the long run, our study might help to develop construction rules for the design of fully synthetic chromosomes.

References
[1] Sobetzko, P., Jelonek, L., Strickert, M., Han, W., Goesmann, A., & Waldminghaus, T. (2016). DistAMo: A Web-Based Tool to Characterize DNA-Motif Distribution on Bacterial Chromosomes. *Frontiers in Microbiology*, *7*, 283. http://doi.org/10.3389/fmicb.2016.00283

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

693/MCBP
Activation of the chaperone Hsp33 by atmospheric pressure plasma
M. Krewing*[1], J. J. Stepanek[1], C. Cremers[2], J. W. Lackmann[1,3], A. Müller[4], B. Schubert[1], P. Awakowicz[3], L. I. Leichert[4], U. Jakob[2], J. E. Bandow[1]
[1]*Ruhr-University Bochum, Applied Microbiology , Bochum, Germany*
[2]*University of Michigan, Ann Arbor, Michigan, United States*
[3]*Ruhr-University Bochum, Electrical Engineering and Plasma Technology, Bochum, Germany*
[4]*Ruhr-University Bochum, Microbial Biochemistry, Bochum, Germany*

Non-thermal plasmas are a promising tool in fields of biomedical and clinical application, as they exhibit several benefits like enhancing wound healing or blood flow. Furthermore, plasmas have decontaminating and disinfecting properties due to the high susceptibility of microorganisms. Plasma interacts with all cellular components. For instance, it introduces modifications into proteins, such as irreversible (over-) oxidation of sulphur-containing amino acids or oxidation of tyrosine and tryptophan residues leading to denaturation and inactivation of enzymes [1].
The impact of plasma on heat shock protein 33 (Hsp33) was investigated using a dielectric barrier discharge known from dermatological applications. Hsp33 as a chaperone responds to oxidative stress and avoids lethal protein aggregation. It is activated by a cysteine oxidation-triggered zinc release and a subsequent unfolding and dimerization [2]. While treatment of proteins with plasma and the accompanied oxidation of certain amino acids usually leads to inactivation, in case of Hsp33 plasma exposure activates the enzyme. To our knowledge, Hsp33 is the first enzyme, which is not inactivated but activated by plasma. The plasma-activation of Hsp33 is reversible indicating the formation of disulphide bonds or sulfenic acids rather than irreversible over-oxidation as observed for other proteins. The reversibility was verified by measuring the redox states of the cysteine residues using thiol-trapping, the protein fold using CD spectroscopy, and protein activity using citrate synthase assay. Application of several scavengers of individual plasma components unrevealed that none of the already identified Hsp33-activating ROS (H_2O_2, HOCl, or hydroxyl radicals) are responsible for plasma-activation of Hsp33.
The identification of the first plasma-activated enzyme that protects cells from protein stress, forces to rethink long-term application of plasma in the clinic. Proteins like Hsp33 could mediate plasma tolerance and provide a basis for resistance development limiting future medical applications.

References
[1] Lackmann, J.-W. *et al*. (2015). *J. Phys. D: Appl. Phys*. 48, 494003.
[2] Cremers, C. M. *et al*. (2010). *J. Biol. Chem.* 285, 11243–11251.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

694/MCBP
Peptidoglycan Structure of *S. carnosus* TM300 - Variations and their Possible Causes
J. Deibert[1], D. Kühner[2,1], M. Stahl[3], E. Koeksoy[4], U. Bertsche*[1]
[1]*University of Tuebingen, IMIT - Microbial Genetics, Tuebingen, Germany*
[2]*University of Tuebingen, IMIT - Infectionbiology, Tuebingen, Germany*
[3]*University of Tuebingen, ZMBP - Analytics, Tuebingen, Germany*
[4]*University of Tuebingen, Applied Geosciences, Tuebingen, Germany*

Peptidoglycan (PGN) is the main component of the bacterial cell envelope. It consists of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) subunits that are linked by β-1,4-glycosidic bonds and, in case of staphylococci, cross-linked through pentaglycine interpeptide bridges.
We isolated the PGN of the non-pathogenic food grade bacterium *S. carnosus* TM300 and analyzed it by UPLC-MS. This resulted in a muropeptide pattern very different from the known one of *S. aureus*. While the PGN of *S. aureus* mainly harbors penta and tetra stem peptides (1), we found the penta peptides in *S. carnosus* to be degraded into tri peptides. In course of our experiments the PGN of *S. carnosus* TM300 turned out to be much more variable than the one of *S. aureus*. We will present data showing that the *S. carnosus* TM300 muropeptide pattern is influenced by genetic as well as by metabolic factors (2).

References
[1] Kühner D, Stahl M, Demircioglu DD, Bertsche U. 2014. From cells to muropeptide structures in 24 h: peptidoglycan mapping by UPLC-MS. Scientific reports 4:7494.
[2] Deibert J, Kühner D, Stahl M, Koeksoy E, Bertsche U. 2016. The Peptidoglycan Pattern of Staphylococcus carnosus TM300—

Detailed Analysis and Variations Due to Genetic and Metabolic Influences. Antibiotics 5:33.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

695/MCBP

anti-YidC antibodies mediated growth inhibition of *S. aureus.*

N. Kumari*[1], M. T. Nguyen[1], F. Götz[1], M. Nega[1]
[1]*Tuebingen University, Microbial Genetics, Tuebingen, Germany*

Biogenesis of membrane proteins requires several processes, including protein translocation, targeting to the membrane, folding and assembling into their protein complexes to function properly in the cell. In bacteria, several pathways are known for protein transfer and membrane insertion. Secretion pathway (Sec-pathway) is a major route of protein translocation in and across cytoplasmic membranes. Membrane protein family YidC/OxaI/Alb3, which is conserved in both prokaryotes and eukaryotes, carries out membrane protein biogenesis with and without sec-machinery.
YidC is a multi-spanning membrane protein and known to be essential for cell viability. Previous studies indicated that in *E. coli* the periplasmic loops are important for its function. Our question is whether anti-YidC antibodies can inhibit the *S. aureus* growth via blocking the enzyme function. For that purpose antibodies were raised specifically against the external loops of *S. aureus* YidC in rabbit.
Our results indicate that YidC loop specific antibodies inhibit the growth of cell wall deficient *S. aureus* at concentration of 100 µg/ml. While two controls of non-immune serum and IgG against a non-essential membrane proteins are not able to inhibit the cell growth at the same concentration. Hence, the external YidC loops seem to be important for the function of this essential enzyme in *S. aureus*.

References

[1] Dalbey, R.E., P. Wang, and A. Kuhn, *Assembly of bacterial inner membrane proteins.* Annu Rev Biochem. 2011;80:161-87. doi: 10.1146/annurev-biochem-060409-092524.
[2] Danese, P.N. and T.J. Silhavy, *Targeting and assembly of periplasmic and outer-membrane proteins in Escherichia coli.* Annu Rev Genet. 1998;32:59-94.
[3] Funes, S., et al., *Evolution of YidC/Oxa1/Alb3 insertases: three independent gene duplications followed by functional specialization in bacteria, mitochondria and chloroplasts.* Biol Chem. 2011 Jan;392(1-2):13-9. doi: 10.1515/BC.2011.013.
[4] Samuelson, J.C., et al., *YidC mediates membrane protein insertion in bacteria.* Nature. 2000 Aug 10;406(6796):637-41.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

696/MCBP

PopZ, a polar landmark, affects cell division and motility in *Magnetospirillum gryphiswaldense*

D. Pfeiffer*[1], M. Toro-Nahuelpan[1], D. Schüler[1]
[1]*Lehrstuhl für Mikrobiologie, Universität Bayreuth, Bayreuth, Germany*

The magnetic bacterium *Magnetospirillum gryphiswaldense* has the ability to align and navigate along the earth magnetic field lines and to swim predominantly towards one of the magnetic poles (swimming polarity). However, the molecular mechanisms which govern magnetotaxis are rather poorly understood [1]. Cells of *M. gryphiswaldense* are apparently symmetrical shaped and swim by means of two flagella (one per pole). Nevertheless, they yet are able to display a distinct magnetotactic swimming polarity. Since the magnetic moment of the magnetosome chain has a fixed orientation (magnetic polarity) with respect to the cellular axis, control of swimming polarity might require a cellular asymmetry such as polar placement of motility related proteins. In search for cellular polarity determinants, in the present study we investigated candidate genes coding for putative landmark proteins. Landmark proteins play important roles in defining cellular polarity in other bacteria such as the related alphaproteobacterial model organism *Caulobacter crescentus* [2]. For example, among others, a gene coding for an orthologue of the PopZ polarity factors was identified in *M. gryphiswaldense*. Deletion of *popZ* resulted in delayed growth, cell elongation and formation of mini-cells as described previously for *C. crescentus*. Overproduction of PopZ resulted in a polar PopZ-rich region mostly devoid of ribosomes and chromosomal DNA. However, PopZ exhibited a bipolar localization pattern throughout the cell cycle in *M. gryphiswaldense* compared to the unipolar to bipolar transition described for *C. crescentus*. A *popZ* deletion mutant was motile, but aerotactic swarm halo formation in semi-solid medium was significantly impaired. Our results suggest that PopZ plays a role as landmark protein in *M. gryphiswaldense.* Deeper knowledge on how cellular polarity is defined could elucidate how the orientation of the magnetic chain dipole and swimming polarity are linked and inherited.

References

[1] Popp, F., Armitage, J. P., & Schüler, D. *Polarity of bacterial magnetotaxis is controlled by aerotaxis through a common sensory pathway.* Nature Communications. 2014. 5, 5398.
[2] Treuner-Lange, A., & Søgaard-Andersen, L. *Regulation of cell polarity in bacteria.* The Journal of Cell Biology. 2014. 206(1), 7–17

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

697/MCBP

The extent of the temperature-induced membrane remodeling in respiratory pathogens *Bordetella pertussis* and *Bordetella bronchiseptica* reflects their adaptation to diverse environmental niches

J. Beranova[1], G. Seydlova[1], I. Bibova[2], A. Dienstbier*[2], J. Drzmisek[2], R. Fiser[1], I. Konopasek[1], B. Vecerek[2]
[1]*Charles University in Prague, Department of Genetics and Microbiology, Prague, Czech Republic*
[2]*Institute of Microbiology v.v.i., Prague, Czech Republic*

Question: Change in the environmental temperature is one of the major stresses faced by microorganisms as it affects the function of the cytoplasmic membrane. Low temperature (below 25 °C) is one of the factors known to attenuate the virulence in closely related respiratory pathogens *Bordetella pertussis* and *Bordetella bronchiseptica.* However, ability to adapt to temperature changes may be different in these bacteria, as *B. pertussis* represents a pathogen strictly adapted to the human body temperature, whereas *B. bronchiseptica* causes infection in a broad range of animals and survives also outside of the host. In this study we have analyzed the thermal adaptation of *B. pertussis* and *B. bronchiseptica* in terms of membrane composition and fluidity as well as virulence factor production.
Methods: For lipid extraction, *B. pertussis* and *B. bronchiseptica* cells were grown in SS medium at 37, 24 and 16 °C. The fatty acid content of the isolated phospholipids was analyzed by coupled GC-MS technology and polar hydrophilic heads composition by thin layer chromatography. Membrane fluidity was analyzed by measuring fluorescence anisotropy of diphenyl hexatriene probe incorporated into liposomes. The expression and production of virulence factors (as well as phosphorylation state of BvgA protein) were determined in cell cultures by qPCR and Western blot, respectively.
Results: In contrast to *B. pertussis*, *B. bronchiseptica* effectively adapted its fatty acid composition to low temperatures. Consequently, the membrane fluidity did not change extensively

in *B. pertussis,* while it was substantially increased in *B. bronchiseptica*. In parallel, the effect of growth temperature changes on the expression and production of selected virulence factors was monitored. Interestingly, while *B. bronchiseptica* switched off the production of virulence factors at low temperature, *B. pertussis* cells displayed constitutive production of these factors. Upon temperature upshift, only *B. bronchiseptica* cells effectively triggered the production of virulence factors and this growth temperature-associated increase correlated with the decrease in fluidity of *B. bronchiseptica* membranes. Observed differential production of virulence factors resulted from different extent of phosphorylation of BvgA transcriptional regulator, which is responsible for the expression of virulence genes.
Conclusions: The striking difference in adaptation to lower temperatures between *B. pertussis* and *B. bronchiseptica* indicates that *B. pertussis* may have lost its ability to modulate the fatty acid composition and thereby to adapt the membrane fluidity. Different extent of adaptation correlated with alterations in the expression and production of virulence factors. We hypothesize that the strongly reduced plasticity of the membrane remodeling results from selective adaptation of *B. pertussis* to the human host.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

698/MCBP
Everything flows and nothing abides: Dynamics of the _Bacillus subtilis_ Min system
H. Feddersen*[1], M. Bramkamp[1]
[1]*LMU Munich, Department 1, Planegg-Martinsried, Germany*

Rod-shaped bacteria like *Escherichia coli* or *Bacillus subtilis* divide with high accuracy at the geometric middle, giving rise to two equally sized daughter cells. This process of binary fission is executed by the divisome, a complex machinery of proteins involved in cell wall synthesis and membrane constriction. The spatial and temporal control of cytokinesis in *B. subtilis* and other bacteria is aided the Min system. The Min system was supposed to prohibit cytokinesis close to the cell poles. The molecular mechanisms of how the Min system localizes the divisome to midcell were analyzed in detail for the *E. coli* MinCDE system. MinE drives a remarkable MinD oscillation in *E. coli* with highest concentration at the poles and a time-resolved minimum at midcell. In contrast, the system in *B. subtilis* was supposed to create a more static and fixed gradient, and consists of the four proteins MinCDJ and DivIVA. Here, DivIVA was supposed to stably tether the MinCD complex to the cell poles, thereby creating a gradient that reduces towards midcell. In previous work we have shown that the Min system in *B. subtilis* is dynamic and relocalizes from the cell poles in non-dividing cells to the divisome during active cytokinesis. Thus, the main site of action seems to be the active divisome.
A detailed understanding of the individual dynamics and localizations of the *B. subtilis* Min components has been lacking, and hence, we set out to investigate the Min dynamics in detail. In order to avoid overexpression artefacts, we first constructed strains in which the individual components (DivIVA, MinJ and MinD) are encoded as functional fluorescent fusion constructs from their native genetic loci. With photoconvertible fluorophores and fluorescence recovery after photobleaching (FRAP) studies we determined Min protein dynamics in growing *B. subtilis* cells. We found that all proteins of the Min system, including the membrane-integral protein MinJ, are highly dynamic. Halftime recovery of the proteins varied from around 7.5s (sfGFP-MinD) up to 73.4s (MinJ-sfGFP), with DivIVA-mNeonGreen recovering half of the protein in around 22s.
To determine putative interactions, we performed the same experiments in knockout backgrounds of the respective non tagged protein(s). DivIVA-mNeonGreen dynamics or localization was not affected in Δ*minJ* and Δ*minCD* backgrounds. MinJ-sfGFP showed a strong decrease in halftime-recovery when examined in a Δ*minD* mutant (29.8s from 73.4s in WT), while the septal localization was only abolished in a Δ*divIVA* background. This points towards a downstream stabilizing effect of MinD on MinJ, while DivIVA is only required for recruitment. sfGFP-MinD in turn required both MinJ directly and DivIVA indirectly for localization. In Δ*minJ* and Δ*divIVA* backgrounds, the fluorescent signal was evenly distributed in the cytoplasm and indicated free diffusion dynamics in FRAP experiments.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

699/MCBP
Effects of membrane depolarization on cell physiology and persister formation in _Escherichia coli_
A. Nolte[1], J. Hoffmann[1], B. Asian[1], G. Wagner[2], B. Berghoff*[1]
[1]*University of Giessen, Institute of Microbiology and Molecular Biology, Giessen, Germany*
[2]*Uppsala University, Department of Cell and Molecular Biology, Uppsala, Sweden*

Clonal populations of bacteria often comprise sub-populations with divergent phenotypic characteristics, a strategy that is often referred to as bet-hedging. It is generally believed that diverse phenotypes guarantee survival under a variety of environmental conditions. The existence of persister cells is one example of such a bet-hedging strategy. Persister cells are viable but non-growing (dormant) cells that appear at very low rates (~0.01%) in exponentially growing populations. They are tolerant (not resistant) to environmental cues, such as antibiotics, and their generation depends – among others – on toxin-antitoxin (TA) systems.
Escherichia coli contains several TA systems, among these the TisB/ IstR-1 system that drives persister formation under DNA-damaging conditions [1]. Transcription of the toxin gene *tisB* is repressed by LexA, the master regulator of the SOS response. When DNA damage occurs, e.g. through the action of antibiotics, *tisB* transcription is induced due to self-cleavage of LexA. However, translation of *tisB* is inhibited by two regulatory RNA elements: the RNA antitoxin IstR-1 [2] and an inhibitory structure in the 5' UTR of *tisB* mRNA [3]. Once TisB toxin is produced, it depolarizes the inner membrane [4], which generates non-growing persister cells due to ATP depletion.
Here, we investigate how cell physiology is changed upon depolarization. We use TisB overexpression and the protonophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP) to induce depolarization. Downstream effects on cell survival, reactive oxygen species production, and activation of stress responses are investigated. To de-repress *tisB* translation, a strain was constructed that is deleted for both inhibitory RNA elements (Δ*istR-1* and Δ1-41 in the 5' UTR of *tisB* mRNA). The strain exhibits stochastic production of depolarized cells and a highly persistent phenotype. The most exciting features of the double deletion strain will be presented and discussed with regard to persister formation in bacteria.

References
[1] Dörr, T., Vulić, M. & Lewis, K. (2010) Ciprofloxacin causes persister formation by inducing the TisB toxin in *Escherichia coli*. *PLoS Biol* 8(2): e1000317.
[2] Vogel, J., Argaman, L., Wagner, E.G. & Altuvia, S. (2004) The small RNA IstR inhibits synthesis of an SOS-induced toxic peptide. *Curr Biol* 14(24): 2271-6.
[3] Darfeuille, F., Unoson, C., Vogel, J. & Wagner, E.G. (2007) An antisense RNA inhibits translation by competing with standby ribosomes. *Mol Cell* 26(3): 381-92.

[4] Unoson, C. & Wagner, E.G. (2008) A small SOS-induced toxin is targeted against the inner membrane in *Escherichia coli*. *Mol Microbiol* 70(1): 258-70.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

700/MCBP

Mutational Analysis of a *nlpD* Gene Affecting Septal Cell Wall Modifications and Cell-Cell Communication in the Filamentous Cyanobacterium *Anabaena* sp. PCC 7120

J. Bornikoel*[1], K. Forchhammer[1], I. Maldener[1]
[1] *IMIT, Organismic Interactions, Tübingen, Germany*

Cell separation in *E. coli* is stimulated by the reversible relief of amidase auto-inhibition through the LytM-domain proteins NlpD and EnvC [1]. Filamentous cyanobacteria like *N. punctiforme* ATCC 29133 and *Anabaena* sp. PCC 7120 do not split into single cells after cell division. Instead they grow in filaments of several hundred cells that are connected by the continuous outer membrane and the common PG layer. The septal PG is perforated by an array of nanopores and it has been demonstrated that periplasmic amidases are involved in the formation of these structures and influence cell-cell communication and cell differentiation [2, 3]. However, the regulation of the specific amidase activity to modulate the septal PG is unknown. To investigate whether LytM proteins are involved in this process, we started the functional characterization of *nlpD* orthologous genes (*alr3353* (*nlpD*1) and *all5163* (*nlpD*2)) in *Anabaena*. Here we describe the phenotype of a *nlpD*1- mutant, a potential regulative protein of periplasmic amidases that influences diazotrophic growth, nanopore array formation and cell-cell communication.

References

[1] Yang, Desirée C., et al. "A conformational switch controls cell wall-remodelling enzymes required for bacterial cell division." *Molecular microbiology* 85.4 (2012): 768-781.
[2] Berendt, Susanne, et al. "Cell wall amidase AmiC1 is required for cellular communication and heterocyst development in the cyanobacterium Anabaena PCC 7120 but not for filament integrity." *Journal of bacteriology* 194.19 (2012): 5218-5227.
[3] Lehner, Josef, et al. "Prokaryotic multicellularity: a nanopore array for bacterial cell communication." *The FASEB Journal* 27.6 (2013): 2293-2300.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

701/MCBP

From binary fission to polar budding: The FtsZ independent cell division of Planctomycetes

C. Boedeker*[1], S. Peeters[2], S. Wiegand[1], M. C. F. van Teeseling[2], T. Kohn[1], R. Mesman[2], M. Rohde[3], L. van Niftrik[2], C. Jogler[1,2]
[1]*Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany*
[2]*Radboud University, Nijmegen, Netherlands*
[3]*Helmholtz Center for Infection Research GmbH, Braunschweig, Germany*

Within the phylum Planctomycetes two different kinds of cell division were observed: typical bacterial binary fission and unusual polar budding. Surprisingly all Planctomycetes -despite this difference- lack the otherwise universal bacterial cell division protein FtsZ. In addition, Planctomycetes lack some of the canonical cell division genes that are part of the *dcw*-operon in other bacteria. While the novel putative cell division ring forming protein kustd1438 was identified in anammox (Anaerobic Ammonium-Oxidizing) Planctomycetes, most other species lack a homolog protein and the molecular background of their cell division remains entirely enigmatic. To ensure cell integrity during separation, peptidoglycan synthesis and cell division are closely linked mechanisms. Thus, the recent discovery of peptidoglycan in Planctomycetes initiated novel possibilities to understand their cell division. This is because to some extent Planctomycetes parallel Chlamydiae, where cell division through budding was recently proposed to be coordinated by MreB instead of FtsZ.
Here we show a novel planctomycetal species, strain Pan216, that was isolated form the volcanic island Panara, Italy. It comprises a sort of intermediate form of cell division, somehow in between binary fission and budding. We revealed the genome sequence of strain Pan216 and compared it against both kinds of Planctomycetes (budding and binary fission) and thereby identified genes putatively involved in the different ways of planctomycetal cell division. Furthermore, we found MreB to be somehow involved in the cell division of various Planctomycetes and employed inhibitors and time-lapse microscopy along with TEM analysis to unearth the role of this protein in planctomycetal cell division.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

702/MCBP

Functional analysis of a bacterial dynamin-like protein DynA

L. Guo*[1], M. Bramkamp[1]
[1]*Ludwig-Maximillians-Universität München, Fakultät Biologie, Planegg-Martinsried, Germany*

Dynamin-like proteins (DLPs) are involved in various membrane remodeling events including in membrane fusion and fission processes in eukaryotic cells. Compared with eukaryotic cells, bacterial DLPs had long been neglected until the first full length structure of a DLP from the cyanobacterium *Noctoc punctiforme* was reported 2006 (Low and Lowe 2006). In recent years, our group has reported that the dynamin-like protein DynA from *Bacillus subtilis* catalyzes membrane fusion in vitro (Burmann, Ebert et al. 2011) and contributes to the innate immunity of bacteria against membrane stress and phage infection(Sawant, Eissenberger et al. 2016). However, so far it is unclear how DynA binds to the bacterial membrane and how the structural details of DynA clusters under membrane stresses are.
In order to uncover the veil of DynA function in vivo, we employed superresolution microscopy (photo-activated localization microscopy, PALM) observing the subcellular localization of DynA. PALM is a widefield imaging method that allows obtaining images with a resolution below the diffraction limit by employing photoswitchable or photoactivatable fluorescent probes. We constructed *B. subtilis* cells expressing DynA fusions with the photoconvertible fluorescent protein Dendra2 for PALM analysis. Furthermore, we are generating a set of mutant proteins to identify the membrane binding region of DynA. The architecture analysis of DynA cluster in vivo and mutation analysis will help us to answer how the dynamin-like protein is assembled and regulated in response to changing environmental conditions.

References

[1] Burmann, F., et al. (2011). "A bacterial dynamin-like protein mediating nucleotide-independent membrane fusion." Mol Microbiol 79(5): 1294-1304.
[2] Low, H. H. and J. Lowe (2006). "A bacterial dynamin-like protein." Nature 444(7120): 766-769.
[3] Sawant, P., et al. (2016). "A dynamin-like protein involved in bacterial cell membrane surveillance under environmental stress." Environ Microbiol 18(8): 2705-2720.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

703/MCBP

Conserved peptides from *Agrobacterium tumefaciens*

A. Kraus*[1], M. Weskamp[1], J. Zierles[1], F. Narberhaus[1]
[1]*Ruhr Universitiy Bochum, Microbial Biology, Bochum, Germany*

The plant pathogen *Agrobacterium tumefaciens* is known to cause crown gall disease in wounded plants by inserting a single stranded piece of DNA into their host. The so-called transfer DNA transports oncogenic genes into the genome of the host causing tumour formation. (1) Despite its widespread biotechnological exploit for plant transfection, the function of many gene products of *A. tumefaciens* still remains unknown (2). Thus *A. tumefaciens* contains five genes coding for hypothetical proteins belonging to the widely distributed, but still uncharacterised DUF1127 family (**d**omain of **u**nknown **f**unction). This conserved domain, usually composed of 40 to 50 amino acids (aa), can be found in several thousand hypothetical proteins from numerous species, mainly bacteria. It either depicts the entire protein or the C-terminus only. (3, 4, 5)

Regarding their length (47/48 aa) and their aa sequence three of five *A. tumefaciens* DUF1127 proteins show a high similarity to each other and to numerous predicted homologues in various other Rhizobiaceae, such as *Rhizobium*, *Sinorhizobium*, *Neorhizobium*, *Ensifer* and other *Agrobacterium* species. The aa composition of *A. tumefaciens* DUF1127 proteins fits to that of an average protein containing a DUF1127 domain. Via Western Blot analysis we could show that the three short hypothetical proteins are synthesised *in vivo*. Additionally a differential expression could be shown for all three genes on transcript level as well as on protein level upon applying different sorts of stress, e.g. heat or cold shock. Despite their high similarity in size and in aa sequence they do not show the same expression pattern, leading to the assumption that they fulfil diverse functions within the cell.

References

[1] Winans SC (1992) Two-way chemical signaling in *Agrobacterium*-plant interactions. *Microbiol Rev* 56(1): 12-31.
[2] Gelvin SB (2003) *Agrobacterium*-mediated plant transformation: the biology behind the "gene-jockeying" tool. *Microbiol Mol Biol Rev* 67**:** 16-37.
[3] Goodner B, *et al*. (2001) Genome sequence of the plant pathogen and biotechnology agent *Agrobacterium tumefaciens* C58. *Science* 294(5550): 2323-8.
[4] Wood DW, *et al*. (2001) The genome of the natural genetic engineer *Agrobacterium tumefaciens* C58. *Science* 294(5550): 2317-23.
[5] Mitchell A, *et al.* (2015) The InterPro protein families database: the classification resource after 15 years. *Nucleic Acids Res*. 43(D1): D213–D21.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

704/MCBP

Cell signaling PII-like proteins; function and structural insights

K. Selim*[1,2], M. Hartmann[1], M. Hartmann[3], K. Forchhammer[2]
[1]*Max Planck Institute for Developmental Biology, Protein Evolution Department, Tübingen, Germany*
[2]*Tübingen University-Interfaculty Institute of Microbiology and Infection Medicine, Organismic Interactions Department, Tübingen, Germany*
[3]*Rostock University, Plant Physiology Department, Rostock, Germany*

PII proteins are widely distributed signaling proteins in nature, and found in all domains of life. Canonical PII proteins bind to different effector molecules (ATP, ADP, 2-OG), the binding induces conformational changes, and enables the PII protein to bind to different targets to modulate different cellular functions. In general, PII proteins are involved in nitrogen metabolism, sensing the cellular energy state with ATP/ADP binding, and sensing C/N state of the cell. All cyanobacteria contain GlnB gene encoding PII homologues. A close examination of available cyanobacteria genomes on CyanoBase revealed further genes with similarity to *glnB* but lacking PII signature sequences, we termed the putative gene products PII-like proteins. The available structural information implies that the PII-like proteins have trimeric-protein structures, which are highly similar to the classical PII core architecture. Thus, it is tempting to speculate that PII-like proteins are involved in regulation of different cellular activities, which differ markedly from classical PII proteins [1-3]. To figure out *in-vivo* cellular function of PII-like proteins, we created different knockout mutants to characterize involvement of PII-like proteins in different physiological functions. The recombinant proteins were titrated with ITC against different effectors molecules to determine the sensory properties of PII-like proteins in comparison to central effector metabolites of classical PII proteins (like, 2-OG). The dynamics of subcellular localization was performed with fusion of PII-like proteins to GFP, and using specific antibodies. Finally, we solved the crystal structures of PII-like proteins for further structure functional analysis.

References

[1] Sant'Anna FH, Trentini DB, de Souto WS, Cecagno R, da Silva SC, Schrank, IS (2009) The PII superfamily revised: a novel group and evolutionary insights. Journal of Molecular Evolution, 68(4), 322-336.
[2] Chellamuthu VR, Alva V, Forchhammer K (2013) From cyanobacteria to plants: conservation of PII functions during plastid evolution. Planta, 237(2), 451-62.
[3] Forchhammer K, Jan Lüddecke J (2016) Sensory properties of the PII signalling protein family. FEBS Journal, 283(3):425-37.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

705/MCBP

The target of an unusual cell wall amidase: Chemical composition of the peptidoglycan from multicellular cyanobacteria of the order *Nostocales*.

E. Bok*[1], M. Borisova[2], C. Mayer[2], K. Forchhammer[1], I. Maldener[1]
[1]*Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Microbiology/Organismic Interaction, Tübingen, Germany*
[2]*Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Bacterial Cell Wall and Glycobiology, Tübingen, Germany*

The N-acetylmuramyl-L-alanine amidase AmiC is a peptidoglycan hydrolase that releases the peptide moiety from N-acetyl muramic acid by cleaving the covalent C-N bond to the N-terminally linked L-alanine residue. It localizes primarily at the septal ring and represents the latest component of the division machinery in *E. coli* (1). The catalytic activity is tightly regulated by NlpD, which removes an α-helix blocking the active site. Filamentous cyanobacteria like *Nostoc punctiforme* or *Anabaena sp. PCC 7120* encode two copies of a homologous *amiC* gene. Instead of cleaving 2 daughter cells, AmiC2 in *N. punctiforme* produces a nanopore array in the septal peptidoglycan and contributes to cell communication and differentiation (2). Detailed structural examinations on the catalytic domain demonstrates that there is no structural component obstructing the activity (3). Hence, AmiC2 of *Nostoc* exhibits a novel function in cell wall modulation and an unknown regulation mechanism. To identify and to characterize this specific role of AmiC orthologues in multicellular cyanobacteria, we started to describe the chemical composition of the cell wall and to investigate the substrate-binding characteristic of AmiC2 by amino acid exchanges in the catalytic side.

Having an outer membrane, cyanobacteria belong to the Gram-negative bacteria, but share common features with Gram-positives like a thicker cell wall and a high cross-linkage (4). Since the chemical composition of filamentous cyanobacteria was unknown,

we performed LC-MS analysis of isolated peptidoglycan from *Nostoc*. First results showed that the sacculus is mainly composed of monomeric and dimeric muropeptides with peptide side chains of 4 amino acids. As in Gram-negative bacteria, the basic structure of the peptidoglycan consists of the carbohydrate backbone components muramic acid, anhydromuramic acid and glucosamine and the amino acids glutamate and meso-diaminopimelic acid.
The results indicate that filamentous cyanobacteria preferably produce a large polymer network of monomeric and dimeric muropeptides. Further experiments have to be performed to determine the degree of cross-linkage and to identify specific restriction sites for AmiC2. Besides, structure and composition of the peptidoglycan from the septal cell wall compared to lateral wall and from different cell types (akinetes, heterocysts and hormogonia) will be investigated to see how the cell wall influences shape and function of specialized cells.

References
[1] Bernhardt et al. (2003). *Molecular microbiology* 48, 1171-1182
[2] Lehner et al. (2013) *FASEB J* 27, 2293-2300
[3] Büttner et al. (2016) *FEBS Journal* 283, 1336-1350
[4] Hoiczyk et al. (2000) *J Bacteriol* 182, 1191-1199

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

706/MCBP
A non-ribosomal peptide synthetase modulates intracellular survival of *Staphylococcus aureus*
S. Blättner[1], A. Moldovan*[1], S. Das[1], M. Krischke[2], T. Hertlein[3], D. Kretschmer[4], M. Müller[2], K. Ohlsen[3], T. Rudel[1], M. Fraunholz[1]
[1]*University of Würzburg, Biocenter, Chair of Microbiology, Würzburg, Germany*
[2]*University of Würzburg, Biocenter, Chair of Pharmaceutical Biology, Würzburg, Germany*
[3]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Georgia*
[4]*University of Tübingen, Infektionsbiologie, Tübingen, Germany*

Staphylococcus aureus is efficiently internalized by human cells and phagosomal escape of *S. aureus* is an essential step for intracellular survival of the bacterium. We screened for virulence factors involved in phagosomal escape by a fluorescent reporter which detects *S. aureus* in the host cell cytoplasm and by using an automated microscopy platform. We thereby identified a function for a staphylococcal non-ribosomal peptide synthetase (NRPS) in phagosomal escape of the pathogen. We show that the pyrazinone NRPS product, the cyclic dipeptide phevaline, is synthesized by intracellular *S. aureus* and we genetically and pharmacologically complemented escape phenotypes in NRPS mutants. Our data suggest that phevalin is required for efficient pathogen survival in neutrophils and macrophages and plays a role in a murine model of pneumonia. Further roles of phevalin in the pathogen-host interaction will be discussed.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

707/MCBP
The new antibiotic rhodomyrtone interacts with phospholipid head groups to generate protein-trapping membrane invaginations
M. Wenzel*[1], D. Saeloh[2,3], V. Tipmanee[4], S. P. Voravuthikunchai[2,3], L. W. Hamoen[1]
[1]*University of Amsterdam, Bacterial Cell Biology, Amsterdam, Netherlands*
[2]*Prince of Songkla University, Department of Microbiology, Hat Yai, Thailand*
[3]*Prince of Songkla University, Excellence Research Laboratory on Natural Products, Hat Yai, Thailand*
[4]*Prince of Songkla University, Department of Biomedical Sciences, Hat Yai, Thailand*

Rhodomyrtone is a promising new antibiotic isolated from the rose myrtle *Rhodomyrtus tomentosa*, a plant used in traditional Thai medicine. While many studies demonstrated its potential in a variety of clinical applications, very little is known about how rhodomyrtone kills bacteria. Preceding studies focused on intracellular targets, such as the cell division protein FtsZ, but could not confirm that rhodomyrtone inhibits intracellular proteins in bacteria *in vivo*. Using live cell microscopy and molecular dynamics simulations, we show that rhodomyrtone targets the bacterial cytoplasmic membrane via a completely novel mechanism. Instead of inserting into the bilayer, rhodomyrtone transiently binds to phospholipid head groups, causing distortion of lipid packing and inducing positive membrane curvature. This leads to invaginations with dramatically increased fluidity, which attract a broad range of membrane proteins. Aberrant localization of these proteins impairs their function, whereby the respiratory chain and the ATP synthase complex were most strongly inhibited.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

708/MCBP
Linked formation and localization of chemotactic signaling arrays by integral array incorporation of a polar determinant
S. Ringgaard*[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Department of Ecophysiology, Marburg, Germany*

Chemotaxis is one of the principal ways bacteria sense, respond, and adapt to changing environmental conditions. The process enables motile bacteria to bias their movement away from unfavorable chemical stimuli and towards more favorable chemical compounds. Chemotaxis proteins organize into large multi-component clusters, usually referred to as chemotactic signaling arrays. The overall structure of chemotaxis arrays is universal, with a highly ordered arrangement between signaling proteins. In *Vibrio* species proper inheritance and localization of signaling arrays to the cell poles is an active process mediated by ParP, which tethers signaling arrays to the polar anchor ParC. While there is much knowledge of array structure, the mechanisms that underlie the formation and localization of these elaborate structures are incompletely understood. Here we have addressed how ParP is able to access chemotaxis proteins within the highly ordered arrays and show that ParP is required for not only array localization but also their formation. In addition to ParPs interaction with the kinase CheA, we identify an essential interaction between ParP and MCP proteins. Particularly, ParP recognizes and interacts with the conserved protein interaction tip of MCP proteins also recognized by CheA and CheW, hence indicating an integral incorporation of ParP into the overall array structure by competing with CheA and CheW for MCP binding. We map interactions between ParP and MCPs and CheA to two distinct regions within a conserved C-terminal domain of ParP, separate from its ParC interaction domain. This allows ParP to simultaneously link two critical signaling components (MCP and

CheA) to the polar anchor ParC. Importantly, the capacity of ParP to interact with both CheA and MCPs, and in consequence its integral incorporation into the array structure, is critical for both the formation and polar localization signaling arrays. Thus, we provide evidence as to how the polar determinant ParP incorporates into the overall array structure, and as an integral part of signaling arrays link the formation of signaling arrays and their polar localization and cellular inheritance.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

709/MCBP

Modulation of the cell wall is important for cell differentiation in the multicellular cyanobacterium *A. variabilis* ATCC 29413

R. Perez*[1], J. Bornikoel [1], I. Maldener[1]
[1]*Universität Tübingen, IMIT/Organismische Interaktionen, Tübingen, Germany*

The planktonic freshwater cyanobacterium *Anabaena variabilis* ATCC 29413 is able to differentiate heterocysts (nitrogen-fixing cells) and akinetes (spore-like cells) to survive under different stress conditions (1). This strain is a model organism to study cell differentiation and is accessible for genetic manipulation. In previous studies we found that modulation of the cell wall is an important aspect of heterocyst and akinete formation, involving cell wall amidases, as AmiC in strains *Anabaena* sp. PCC 7120 and *Nostoc punctiforme* (2,3,4). Here we present the functional characterization of three cell-wall related proteins in *A. variabilis* and showed that they are important for heterocyst and akinete differentiation. The GerM-domain protein, encoding by the *Ava_2312* gene of *A. variabilis*, contains a large periplasmic domain, which shows homology to a sporulation protein from *Bacillus subtilis* (5). An Δ*Ava_2312* mutant showed a severe filament dystrophy with aseriate filaments, which was not able to differentiate heterocysts and mature akinetes. A different phenotype was obtained by mutation of genes *Ava_1465* and *Ava_1466* encoding cell wall amidases AmiC1 and AmiC2 from *A. variabilis*. These mutants showed elongated vegetative cells and a massive deposition of peptidoglycan in the cell wall. Upon nitrogen starvation they formed aberrant heterocysts and were not able to grow diazotrophically. In all three cell-wall mutants, deposition of a glycolipid layer in the special envelope of akinetes was aberrant. Their akinetes were less resistant towards lysozyme and showed lower survival rates compared to the wild type. This study confirms the importance of cell wall modulation for differentiation in multicellular cyanobacteria.

References

[1] Perez R, Forchhammer K, Salerno G, Maldener I. (2016) Clear differences in metabolic and morphological adaptations of akinetes of two Nostocales living in different habitats. Microbiology. 162:214-23.
[2] Lehner J, Zhang Y, Berendt S, Rasse TM, Forchhammer K, Maldener I.(2011) The morphogene AmiC2 is pivotal for multicellular development in the cyanobacterium *Nostoc punctiforme*. Mol. Microbiol. 79:1655-69.
[3] Berendt S, Lehner J, Zhang YV, Rasse TM, Forchhammer K, Maldener I. (2012) Cell wall amidase AmiC1 is required for cellular communication and heterocyst development in the cyanobacterium *Anabaena* PCC 7120 but not for filament integrity. J. Bac.194:5218-27.
[4] Lehner J, Berendt S, Dorsam B, Perez R, Forchhammer K, Maldener I. (2013) Prokaryotic multicellularity: a nanopore array for bacterial cell communication. FASEB J. 27:2293-300.
[5] Slynn GM, Sammons RL, Smith DA, Moir A, Corfe BM. (1994) Molecular genetical and phenotypical analysis of the gerM spore germination gene of *Bacillus subtilis* 168. FEMS Microbiol. Lett. 121:315-20.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

710/MCBP

Maturation of the *Salmonella* containing vacuole is dependent on the host cell cycle

C. Lisowski*[1], C. Maudet[1], A. R. Cruz[2], M. Mano[2], A. Eulalio[1]
[1]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*
[2]*University of Coimbra, Center for Neuroscience and Cell Biology, Coimbra, Portugal*

Introduction: *Salmonella* Typhimurium pathogenicity depends on its ability to enter both phagocytic and non-phagocytic cells, in particular gut epithelial cells, and to replicate within membrane bound vacuoles, known as *Salmonella* containing vacuoles (SCVs). Invasion and intracellular replication of *Salmonella* rely on two distinct type III secretion systems (T3SS), encoded on pathogenicity island 1 and 2 (SPI-1 and SPI-2, respectively). The effector proteins translocated through the SPI-2 T3SS, which is induced in the acidic environment of SCVs, are involved in SCV maturation, *Salmonella* intracellular survival and replication.
Our laboratory has recently identified microRNAs (miRNAs) as important regulators of *Salmonella* infection. Among the strongest inhibitors of *Salmonella* infection, we identified miRNAs that hinder G1/S cell cycle progression, uncovering a crucial role of the host cell cycle during infection.
In this study we aimed at characterizing the interplay between the host cell cycle and *Salmonella* infection, specifically at determining why *Salmonella* replication is impaired in host cells in the G1 phase of the cell cycle.
Results: We show that arresting host cells in G1 phase of the cell cycle inhibits *Salmonella* intracellular replication, without interfering with invasion into host cells. Using confocal and transmission electron microscopy, we reveal that cells in G1 phase have altered endosomal trafficking, displaying an accumulation of lysotracker and LAMP1 positive vesicles, but exhibiting a decreased activity of lysosomal enzymes. Moreover, the maturation of the SCV was impaired in G1 cells. By using *Salmonella* SPI-2 reporter strains and qRT-PCR, we show that G1 cells present a deficient activation of *Salmonella* SPI-2 T3SS. In agreement with limited SCV maturation, cytoplasmic replication of *Salmonella* is favored in G1 cells. Transfecting cells with mimics of miRNAs that block G1-S progression resulted in impaired *Salmonella* SPI-2 T3SS activation. Interestingly, the expression of these miRNAs is decreased upon *Salmonella* infection.
Conclusion: Overall, our results show that *Salmonella* replication is inhibited in cells in the G1 phase of the cells cycle due to impairment in the maturation of the *Salmonella* containing vacuole. Interestingly, *Salmonella* prepares the intracellular environment for its replication by controlling the host cell cycle, in part by regulating the expression of host miRNAs. *Salmonella* infection leads to faster transition between the G1 and S phases of the cell cycle through the decreased expression of certain miRNAs (e.g. miR-15 family). This enforces a faster progression into G2 phase of the cell cycle, which is favorable for *Salmonella* replication.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

711/MCBP

Genetic Control of the Formation of Multicellular Aggregates in *Staphylococcus aureus*

C. Wermser*[1], D. Lopez[1,2]
[1]*Uni Würzburg, Research Center for Infectious Diseases, Würzburg, Germany*
[2]*Universidad Autonoma de Madrid, Centro Nacional de Biotecnologia, Madrid, Spain*

Sessile multicellular communities like biofilms are the predominant mode of microbial life. Its ability to form biofilms on biotic and abiotic surfaces such as catheters, implants and tissues has made the opportunistic pathogen *Staphylococcus aureus* a model organism to study biofilm-related processes. Studies using submerged *S. aureus* biofilms have formed the current wide-ranging knowledge on the complex pathways and regulators that influence biofilm formation. It has become apparent that *S. aureus* possesses versatile strategies for efficient biofilm formation that are strongly strain- and context-dependent.
An alternative *in-vitro* approach to study biofilm formation has previously been established in our lab[1]. The macrocolony biofilm-like developmental model mimics the conditions of bones, joints and soft tissues on a solid agar surface. The model allows for in-depth studies of community-wide processes related to microbial evolution and bacterial cell differentiation. We employed a screen of a genome-wide transposon library[2] to determine novel determinants of macrocolony biofilm morphology. Several previously undescribed determinants were characterized regarding their roles in macrocolony morphology development. The results that will be presented outline the signaling processes and metabolic requirements that lead to the complex architecture of these multicellular communities. Additionally, we gained insight in the involvement of the novel determinants in the core signaling processes that control the development of multicellular communities. With this work, we expand the current understanding of biofilm formation and contribute to the comprehensive characterization of an alternative developmental model to study biofilm formation.

References

[1] Koch G, Yepes A, Forstner KU, et al. Evolution of Resistance to a Last-Resort Antibiotic in Staphylococcus aureus via Bacterial Competition. Cell 2014;158:1060-71.
[2] Fey PD, Endres JL, Yajjala VK, et al. A genetic resource for rapid and comprehensive phenotype screening of nonessential Staphylococcus aureus genes. mBio 2013;4:e00537-12.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

712/MCBP

Mechanism of activation of the two-component system KdpD/KdpE by the accessory protein PtsN

M. Mörk-Mörkenstein*[1], R. Heermann[2], Y. Göpel[1], K. Jung[2], B. Görke[1]
[1]*Max F. Perutz Laboratories, University of Vienna, Vienna, Austria*
[2]*Ludwig-Maximilians-University, Munich, Germany*

The nitrogen-related phosphotransferase system (PTSNtr) is a phospho-relay, which consists of the proteins PtsP, PtsO and PtsN, and works in parallel to the canonical (transport) PTS in *Escherichia coli*.[1] Previously, PTSNtr was shown to play a role in potassium homeostasis: PtsN stimulates the expression of the high affinity potassium transporter KdpFABC by affecting the activity of the two component system KdpD/E. [2] The sensor kinase KdpD auto-phosphorylates upon potassium starvation and in turn phosphorylates the response regulator KdpE. Phosphorylated KdpE binds the *kdp* promotor and activates expression. Dephosphorylated PtsN was shown to bind and stimulate auto-phosphorylation of KdpD.[2,3] However, many questions how PtsN influences KdpD still remained. In this project, we investigated the mechanism underlying interaction of KdpD and PtsN. Both, PtsN and KdpE bind to the HisKA domain of KdpD. Using a two-hybrid system and surface plasmon resonance measurements we show that KdpE and PtsN compete for binding to KdpD. To our knowledge this is the first example of an activating accessory protein binding directly to the HisKA domain of a Histidin kinase. However, KdpE displayed a much higher affinity for KdpD as compared to PtsN. On first glance, these observations seem contradictory: On the one hand PtsN stimulates the activity of the histidine kinase KdpD, on the other hand it blocks the access of response regulator KdpE to KdpD. However, it is known that the histidine kinase forms a dimer and that one monomer is phosphorylated by the second monomer in trans. Our data indicate a model, in which the accessory protein binds to one monomer of the histidine kinase stimulating phosphoryl-group transfer to the second histidine kinase monomer, which then phosphorylates the response regulator.

References

[1] Pflüger-Grau and Görke, 2010, Trends Microbiol. 18(5): 205-214.
[2] Lüttmann et al., 2009, Mol. Microbiol. 72(4): 978-994.
[3] Lüttmann et al., 2015, J. Mol. Microbiol. Biotechnol. 25(2-3): 168-177.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

713/MCBP

***Bacillus subtilis* surfactin influences *Aspergillus niger* morphology and activates the cell wall integrity pathway**

A. Richter*[1], M. Arentshorst[2], J. Schwitalla[1], S. Bhat[1], F. Blei[1], A. F. J. Ram[2], C. A. M. van den Hondel[2], A. T. Kovacs[1]
[1]*Friedrich Schiller University Jena, Institute of Microbiology, Terrestrial Biofilms Group, Jena, Germany*
[2]*Leiden University, Institute of Biology Leiden, Microbial Biotechnology, Leiden, Netherlands*

Bacteria and fungi can coexist and interact in nature in various forms spanning from symbiosis to producing antimicrobial compounds that impair or kill the other organism[1]. Previous study on *B. subtilis* and *A. niger* interaction revealed that genes involved in metabolism and putative antimicrobial production are differentially expressed upon close contact of the two organisms[2].
Here, we examined the morphological changes of fungal hyphae that were observed during co-cultivation of *B. subtilis* with *A. niger*. We were interested which bacterially produced compound is responsible for fungal cell shape alteration and how the cell biology of *A. niger* is altered.
A. niger strains (wild type, cell organelle-reporters, or various mutants) were co-cultivated with supernatant of *B. subtilis* NCIB 3610 or its mutant derivatives lacking the ability to produce selected secondary metabolites. Morphology changes and cell organelles were visualized using confocal microscopy. Subsequently, activation of cell wall integrity pathway was measured using a luminescence reporter gene construct and the activity of this reporter was measured during incubation with supernatant of *B. subtilis* NCIB 3610 or its mutant derivative.
Incubation of *A. niger* with supernatant of *B. subtilis* culture causes severe morphological changes in the fungal hyphae leading to a blown up, rounded structure of the growing hyphae, which we termed "bulbous cells". Supernatant of a surfactin mutant of *B. subtilis* does not induce bulbous cell formation. Cell organelle reporters showed that secretory vesicles are miss-localized in the presence of surfactin. Interestingly, bulbous cells are more abundant in *A. niger* Δ*rlmA* strain. RlmA is a part of the cell wall integrity pathway, where cell wall stress activates a signaling cascade leading to production of the transcription factor RlmA, which in turn induces cell wall biosynthesis and remodeling. A

reporter strain able to measure cell wall stress induction via the RlmA cell wall integrity pathway was used and showed increased cell wall stress, when *A. niger* is exposed to supernatant of *B. subtilis* wild type. This is not the case with the surfactin mutant strain.
Surfactin was identified as the predominant agent causing the morphological changes in fungal hyphae during co-cultivation with *B. subtilis*. The cell wall integrity pathway of *A. niger* was found to be activated when exposed to *B. subtilis* surfactin.

References
[1] Frey-Klett et al., 2011, Bacterial-Fungal Interactions: Hyphens between Agricultural, Clinical, Environmental, and Food Microbiologists. *Microbiol. Mol. Biol. Rev.* 75: 583-609.
[2] Benoit et al., 2015, *Bacillus subtilis* attachment to *Aspergillus niger* hyphae results in mutually altered metabolism. *Environ Microbiol* 17: 2099-2113.

Presentation on Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

714/MCBP
***Neisseria meningitidis* Opc and type IV pili target host sphingolipid metabolism**
J. Becam[1], S. Hebling[1], A. Simonis[2], A. Burgert[3], M. Sauer[3], A. Schubert-Unkmeir*[1]
[1]*Institute of Hygiene and Microbiology, Wuerzburg, Germany*
[2]*University Hospital Zurich, Division of Hematology, Zuerich, Switzerland*
[3]*University Wuerzburg , Department of Biotechnology & Biophysics, Würzburg, Germany*

Question: *Neisseria meningitidis* (*Nm*), an obligate human pathogen, is a causative agent of septicemia and meningitis worldwide. The interaction with brain endothelial cells is central to the pathogenicity of meningococcal meningitis. Recent studies demonstrated that distinct ceramide-rich membrane domains are important in this process.
Ceramide can be generated via the salvage pathway through the action of sphingomyelinases, or the *de novo* synthetic pathway through the action of ceramide synthases. Due to their biophysical properties, ceramide-rich membrane domains fuse into extended ceramide-rich platforms (CRPs) which span a few hundred nanometers to several micrometers and serve to sort and concentrate membrane receptors. The aim of the study was to identify meningococcal factors activating the acid sphingomyelinase (ASM), which induces the formation of CRPs during the process of meningococcal adhesion to and invasion into brain endothelial cells.
Methods: We employed human brain microvascular endothelial cells (HBMEC) as an *in vitro* model to analyse whether *Nm* stimulates surface ceramide display on brain endothelial cells. The role of ASM and ceramide-rich domains was analysed using flow cytometry, enzyme assays and confocal immunofluorescence microscopy. In addition, CRPs were characterized by *direct* stochastic optical reconstruction microscopy (*d*STORM). In order to identify meningococcal factors responsible for activating the ASM/ceramide system isogenic meningococcal mutants and complemented strains were constructed.
Results: We show that *Nm* causes transient activation of ASM followed by ceramide release in brain endothelial cells. In response to *Nm* infection, ASM and ceramide are displayed at the outer leaflet of the cell membrane and condense into large CRPs which also concentrate the ErbB2 receptor. We already showed that the outer membrane protein Opc is required for *Nm*-mediated ASM activation. In addition, we could now show that meningococcal type IV pili also significantly contribute to ceramide release: loss-of-function-mutation in the major pilin protein (*pilE*) or the minor pilin proteins (*pilV*, *pilX*, *comP*) led to significant less ceramide levels on HBMEC as determined by flow cytometry analysis. Depletion of intracellular Ca2+ by cell-permeable EGTA-AM inhibited ASM translocation to the outer leaflet and formation of CRPs and suggested that type IV pili triggered ASM translocation and ceramide release in a Ca2+ dependent manner via lysosomal exocytosis.
Conclusions: Our results demonstrate that both the outer membrane protein Opc and type IV pili contribute to activation of the ASM/ceramide system by the species *Nm* determining its invasiveness into brain endothelial cells.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

715/MCBP
Identification of lytic proteins that potentially mediate predatory behavior in *Myxococcus xanthus*
K. Arend[1], T. Bentler[1], C. Kaimer*[1]
[1]*Ruhr-Universität Bochum, Bochum, Germany*

The gram-negative soil bacterium *Myxococcus xanthus* displays characteristic behavior patterns, like group motility and the formation of multicellular fruiting bodies. *M. xanthus* is also a bacterial predator that thrives on the targeted lysis of other microorganisms and the consumption of the released macromolecules (1).
Although predation has been observed decades ago, the regulation of predatory behavior and the molecular mechanisms that mediate prey recognition and prey lysis remain largely unknown. Previous studies have shown that *M. xanthus* predation requires regulated cell motility and involves an antibiotic secondary metabolite (1, 2). The production of bacteriolytic enzymes by *M. xanthus* has been reported, but the proteins and corresponding genes have not been characterized (3).
In order to understand *M. xanthus'* predation on a molecular level, we aim to identify lytic factors involved in the disintegration of bacterial prey cells, and to study their functional mechanism and regulation. We combined zymography methods, using different bacterial substrates, with LC-MS to identify potential lytic proteins in *M. xanthus* cell extract and in secreted proteins. First candidates are under investigation and induce cell lysis when expressed heterologously in *E. coli*.

References
[1] Keane, R & Berleman, JE (2016): The predatory life cycle of *Myxococcus xanthus*. Microbiology 162: 1-11
[2] Xiao, X *et al*. (2011) Antibiotic production by myxobacteria plays a role in predation. J. Bact. 193: 4626-33
[3] Sudo, S & Dworkin, M (1972): Bacteriolytic enzymes produced by *Myxococcus xanthus*. J. Bact. 110: 236-45

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

716/MCBP
Development of an improved primary in vitro model of the human small intestine
M. Schweinlin[1], M. Schnurr*[1], S. Wilhelm[1], H. Walles[1,2], M. Metzger[1,2]
[1]*University of Wuerzburg, Department of Tissue Engineering and Regenerative Medicine (TERM), Würzburg, Germany*
[2]*Würzburg branch of the Fraunhofer Institute Interfacial Engineering and Biotechnology (IGB), Translational Center Würzburg "Regenerative Therapies for Oncology and Musculoskeletal Diseases" (TZKME), Würzburg, Germany*

Introduction: Studying host-pathogen encounters requires appropriate models that properly reflect the complexity of these interactions. While small animal models have been invaluable for our understanding of the biology of infection, many pathogens are either unable to infect these model organisms or they do not fully recapitulate the observed pathogenesis in humans. To overcome these issues, we have developed a 3D in vitro tissue model of the

human small intestine that mimics the infection-relevant physiological organ context.
Objectives: In our study, we applied primary human intestinal epithelial cells in a 3D in vitro culture system in order to mimic the microenvironment of the gut in vivo.
Materials & methods: Intestinal crypts including stem cells were isolated from human small intestinal tissue samples and co-cultured on a decellularized porcine gut matrix together with intestinal fibroblasts. In vitro models were maintained under static and dynamic conditions for 7 days. Epithelial integrity was tested by FITC-dextran (4kDa) and TEER-measurement. Models were further characterized by qPCR, immunohistochemistry, electron microscopy and transport assays.
Results: Intestinal cells have formed a monolayer including all the differentiated cell types shown by, Mucin2, Villin, Chromogranin A, and Lysozyme immunohistochemistry. Electron microscopy depicted essential functional units of an intact epithelium such as microvilli and tight junctions. FITC-dextran and TEER-measurement proved tightness of the cell layer. Models showed characteristic transport activity for several reference substances.
Conclusion: As previously demonstrated for several culture systems, the development of intestinal in vitro systems based on human primary cells instead of using cell lines more closely reflects the in vivo situation. Therefore, our small intestinal model provides a very promising tool not only for the significant reduction of animal experiments, but also as a more reliable preclinical test platform to study the bacteria-host interactions.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

717/MCBP
Interaction of prophages and colicin Ib at the single cell level in *Salmonella enterica* serovar Typhimurium
S. Spriewald*[1], J. Müller[2], B. Hense[3], N. Obeng[4], B. Stecher[1]
[1]*Max von Pettenkofer Institute, Munich, Germany*
[2]*Technische Universität München, Centre for Mathematical Sciences, Garching, Germany*
[3]*Helmhotz Zentrum München, ICB Institute of Computational Biology, Neuherberg, Germany*
[4]*Christian-Albrechts-Universität zu Kiel, Kiel, Germany*

Colicins are protein toxins produced by and toxic for membes of the Enterobacteriaceaefamily (i.e. *E. coli*, Salmonella spp.). Colicins are only expressed by a fraction of the (genetically identical) population (phenotypic heterogeneity). Upon colicin release, bacteria are lysed, while the remaining part of the population survives and gains a fitness advantage against colicin-sensitive competitors. This scenario is referred as *division of labor* and can eventually increase the overall fitness of a colicin-producer in complex ecosystems. Importantly, colicins can be subdivided in two groups (A and B). Among other differences, group A colicins encode for a cognate lysis protein and release colicin by lysis protein-mediated cell lysis. In contrast, group B colicins do not encode for a cognate lysis protein and the release mechanism of group B colicins remained unclear. We characterized expression and release of pore-forming group B colicin Ib (ColIb) by the human enteric pathogen *S.* Typhimurium SL1344 (*S.* Tm). We could show that ColIb (*cib*) confers a fitness benefit to *S.* Tm in competition against ColIb susceptible *E. coli* strains in mouse model for *S.* Tm induced colitis. Moreover, we recently demonstrated that, in contrast to group A colicins, ColIb is released in the course of temperate phage cell lysis. Our data reveal a new mechanism for colicin release and point out a novel function of temperate phages in enhancing colicin-dependent bacterial fitness. Here, we characterized *cib* expression and temperate phage-mediated cell lysis at the single cell level using *gfp*- and *rfp*- based fluorescent protein reporter tools, respectively. Using these reporters we could demonstrate by single cell analysis (i.e. FACS, microscopic image analysis and live cell microscopy) that *cib* expression and activation of the lysis genes of temperate phages are coregulated within individual bacteria.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

718/MCBP
First in – first out: Phenotypic memory couples entry and exit from bacterial dormancy in *Bacillus subtilis*
A. Mutlu*[1,2], M. Ziesack[2], S. Trauth[1,2], I. Bischofs[1,2]
[1]*MPI for Terrestrial Microbiology, Marburg, Germany*
[2]*University of Heidelberg, Center for Quantitative Analysis of Cellular and Molecular Biosystems (BioQuant), Heidelberg, Germany*

Bacteria withstand starvation by forming dormant spores that revive when nutrients become available. Although sporulation and spore revival jointly determine survival in fluctuating environments, the relationship between them has been unclear.
Here we developed an advanced time-lapse microscopy assay and a fluorescent marker that reports on each spores differentiation history to study the effect of variable sporulation timing on nutrient-induced spore revival.
We show that heterochronous sporulation leads to the formation of distinct spore subpopulations. *Early* spores were kinetically favored over *late* spores on different levels of the revival pathway and in response to different stimuli. Spores exhibit long-term phenotypic memory of their differentiation history and we identify alanine dehydrogenase as an important contributor to this memory.
Our results add to growing body of evidence that cellular memory plays an important role in microbial stress responses. We provide a simple model for how phenotypic memory shapes bacterial dormancy which could very well apply to other species and help us to understand and ultimately control complex microbial behaviors.

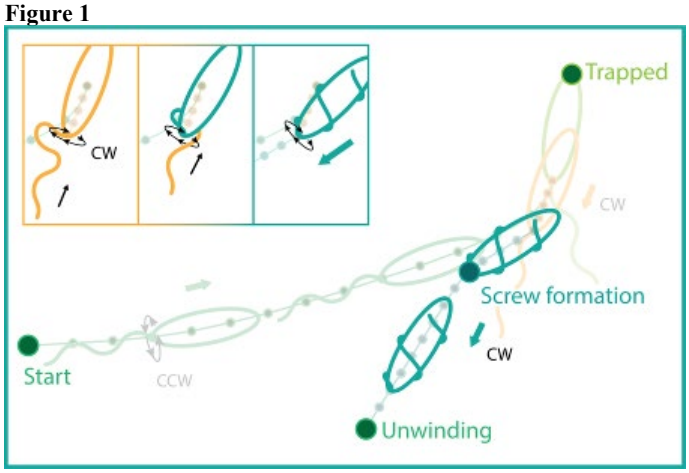
Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

719/MCBP
Flagellar instability-driven escape mechanism for trapped bacteria
M. Kühn*[1], F. Schmidt[2], B. Eckhardt[2], K. Thormann[1]
[1]*Justus-Liebig University, Department of Biology, Gießen, Germany*
[2]*Philipps-University, Department of Physics, Marburg, Germany*

Many bacteria are motile by means of a single flagellum at the cell pole which has a rather rigid helical shape allowing forward and backward swimming in liquid media. However, many natural bacterial habitats consist of structured environments, such as sediments, water-saturated soils, or tissues, where bacteria run the risk of getting stuck. Using high-speed microscopy, we monitored the swimming behavior of the mono-polarly flagellated species *Shewanella putrefaciens* with fluorescently labeled flagellar filaments. To mimic the conditions of its natural habitats we observed single cells at an agarose-glass interface featuring both wide liquid filled channels and constricted areas. We found that when cells get stuck and regular backing out does not release the cell, they use a yet undescribed movement: the flagellar filament wraps around the cell body in a spiral-like fashion, enabling the cells to escape by a screw-like backward motion. Microscopy and modeling suggest that this propagation mode is triggered by an elastic instability at the base of the filament upon switching to clockwise motor rotation and when higher torque is applied. The switch is reversible, so cells can return to regular swimming mode by switching motor direction back to counterclockwise rotation. An instability of the flagellar structure can be employed for a novel rescue and movement mechanism and is likely found in numerous environmental and pathogenic bacteria.

Figure 1: Illustration of trapping, screw formation and escape of a swimming cell in a constricted environment based on high-speed fluorescence microscopy. The cell enters from the left, swims until it gets stuck at the upper right and tries to get out by swimming backward. Then the flagellar instability sets in, the flagellum wraps around the cell body (see inset) and the bacterium escapes in a screw-like motion toward the lower half of the frame.

Figure 1



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

720/MCBP

Single-cell quantification of ribosomes with super-resolution microscopy

S. Matamouros*[1], T. Gensch[2], I. Abdollahzadeh[2], C. Sachs[1], J. Hendriks[2], K. Nöh[1], M. Bott[1]
[1]*Forschungszentrum Juelich, IBG-1, Juelich, Germany*
[2]*Forschungszentrum Juelich, ICS-4, Juelich, Germany*

Corynebacterium glutamicum is an important industrial biotechnology model organism. We intend to improve its growth rate through rational genome engineering. In bacteria, 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 protein-RNA complexes that make up a significant fraction of the cellular RNA and protein content. Ribosome numbers must be, therefore, tightly controlled since an excess can lead to a decrease in cellular efficiency due to the misallocation of resources with a consequent fitness reduction for the bacteria. To understand if growth rates can be improved by altering ribosome content in *C. glutamicum* we tested strains containing extra rRNA copies and determined ribosome abundance in cells cultivated at different growth rates. We show that additional rRNA copies allow cells to faster adapt to different growth conditions. Furthermore, via single-cell super-resolution microscopy and biochemical assays we confirm that there is a direct correlation between growth rate and cellular ribosomal content.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

721/MCBP

Minor pilins & PilY1 proteins in type IV pili-dependent motility in Myxococcus xanthus

A. Treuner-Lange*[1], Y. W. Chang[2], G. J. Jensen[2], L. Søgaard-Andersen[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*
[2]*California Institute of Technology, Pasadena, California, United States*

Type IV pili (T4P) are filamentous cell surface structures and are highly dynamic undergoing cycles of extension, adhesion and retraction. We recently elucidated the architecture of the T4P machine in M. xanthus and mapped the 10 core proteins of the T4P machine to this architecture using electron-cryotomography (ECT) in combination with an informative set of mutants and fluorescent fusion proteins. Moreover, we have proposed a detailed model for the overall structure and function of this machine [1].

In addition to the 10 core proteins of the T4P machine, the so-called minor pilins and PilY1 are important for T4P function. Minor pilins have been reported to be present in small amounts in the T4P fiber and have been suggested to prime pilus assembly and/or counteract retractions. PilY1 proteins are described as pilus-associated adhesins and are suggested to function in adhesion and/or anti-retraction and/or mechanosensing.

To understand the function of minor pilins and PilY1 inT4P biology, we identified the corresponding genes in M. xanthus. We identified three gene clusters each with four minor pilin genes and one *pilY1* gene. The corresponding proteins were named FimU-PilV-PilW-PilX_1-3, and PilY1_1-3 following the nomenclature of the homologous proteins of Pseudomonas aeruginosa.

To analyze the function of the minor pilins and PilY1 proteins of *M. xanthus* we systematically deleted minor pilin and/or pilY1 genes in the genome and analyzed for T4P-dependent motility and T4P formation in the mutants. While deletion of genes of cluster_1 or _3 did not affect T4P-dependent motility, lack of genes from both clusters or all three clusters abolished T4P-dependent motility and T4P formation indicating that cluster_1 or cluster_3 are sufficient to support T4P-dependent motility and T4P formation.

Using ECT on intact cells of a nine-fold minor pilin mutant as well as the triple pilY1 mutant, we only detected non-piliated T4P machines and these empty machines lacked a short periplasmic stem structure that is present in non-piliated T4P machines in wild-type and connects to the inner membrane.

We conclude (1) that minor pilins and PilY1 proteins are essential for T4P formation, (2) that the short stem functions as a priming complex for T4P assembly and (3) that the major pilin PilA, the minor pilins and PilY1 proteins are part of this complex.

References

[1] Chang Y-W, Rettberg L, Treuner-Lange A, Iwasa J, Søgaard-Andersen L, Jensen G J (2016) Structure of the type IVa pilus machine in vivo. Science 351:aad2001

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

722/MCBP

Characterization of small proteins in *E. coli* and *Salmonella*

E. Venturini*[1], A. J. Westermann[1], L. Barquist[1], L. Li[2], J. Hör[1], J. Vogel[1]
[1]*Institute for Molecular Infection Biology, University of Würzburg, Würzburg, Germany*
[2]*Baylor College of Medicine, Division of Biostatistics, Dan L. Duncan Cancer Center, Houston, Texas, United States*

Small proteins in bacteria have been underrepresented since the beginning of the whole-genome annotation era, as their size makes both their prediction and their investigation with standard biochemical approaches challenging. Information regarding their presence has been slowly building up in the recent years, and functional characterization demonstrated that they play important

roles in diverse cellular processes: from regulation of translation to regulation of the activity of protein complexes, inducing post-segregational killing and stress responses, and many others.
The Gram-negative bacteria *E. coli* and *Salmonella* are two well-established model organisms. While the first is probably the best characterized non-pathogenic bacterium, the other is a model for host-pathogen interaction as it can establish infection in a variety of hosts. In both cases, small proteins shorter than 100 aa account for approximately 10 % of the currently annotated proteome. On one hand, ribosomal proteins, members of toxin-antitoxin systems and leader peptides are examples of well described small proteins; on the other hand, around 50 % remains uncharacterized.
Here we integrate global data on the expression, regulation, biochemical behavior, and loss-of-function phenotypes of bacterial small proteins. That is, we inspected (1) dual RNA-seq data to identify *Salmonella* small proteins that are expressed and regulated during host infection, (2) transposon-directed insertion sequencing (TraDIS) to reveal the consequence of their inhibition for infection, and (3) gradient profiling, that informs about the involvement of small proteins in larger complexes, both in *Salmonella* and *E. coli*. We complemented these experimental data with results from a recently developed computational pipeline for the prediction of small proteins, called sPepfinder. In doing so, we will identify novel bacterial small proteins and we may obtain first insights into their cellular functions, likely providing new information about bacterial physiology and pathogenesis.

References
[1] Westermann, A. J., Förstner, K. U., Amman, F., Barquist, L., Chao, Y., Schulte, L. N., Müller, L., Reinhardt, R., Stadler, P. F., & Jörg Vogel. Dual RNA-seq unveils noncoding RNA functions in host–pathogen interactions. *Nature*. 529, 496–501 (2016).
[2] Barquist, L., Langridge, G. C., Turner, D. J., Phan, M. D., Turner, A. K., Bateman, A., Parkhill, J., Wain, J., Gardner, P.P. A comparison of dense transposon insertion libraries in the *Salmonella* serovars Typhi and Typhimurium. *Nucleic Acids Res*. 41, 4549-64 (2013)
[3] Smirnov, A., Förstner, K. U., Holmqvist, E., Otto, A., Günster, R., Becher, D., Reinhardt, R., Vogel, J. Grad-seq guides the discovery of ProQ as a major small RNA binding protein. *Proc Natl Acad Sci U S A*. 113, 11591–11596 (2016).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Microbial Diversity and Evolution (FG MDE)

723/MDEP
A new *Rhizobium* species with a high intra-species diversity isolated from the permanent grassland of the environmental monitoring and climate impact research station in Linden, Giessen, Germany.
E. L. Aydogan*[1], H. J. Busse[2], G. Moser[3], C. Müller[3], P. Kämpfer[1], S. P. Glaeser[1]
[1]*Justus-Liebig-University, Institute of Applied Microbiology, Giessen, Germany*
[2]*University of Veterinary Medicine Vienna, Institute of Bacteriology, Mycology and Hygiene , Vienna, Austria*
[3]*Justus-Liebig-University Giessen, Institute for Plant Ecology, Giessen, Germany*

Many species of the genus *Rhizobium* have been isolated from various plant organs and plant species and show a broad range of beneficial effects on plants. While studying phyllosphere inhabiting bacteria of two abundant plant species, *Arrhenatherum elatius* and *Galium album,* of the permanent grassland of the research area in Linden, Germany, abundant 16S rRNA gene sequence identical isolates were identified as *Rhizobium* spp. The isolates were isolated and cultured from leaves of both plants at different time points collected from the permanent grassland of the Giessen Free air carbon dioxide enrichment (Gi-FACE) system grown under ambient or elevated CO2 (+ 20 %) concentrations and from a warming experiment with ambient plots and (+2 °C) surface temperature elevated plots from the same field site. Phylogenetic analyses based on the nearly full length 16S rRNA gene and nucleotide and amino acid sequences of protein coding housekeeping genes (*recA, atpD*) showed that the isolates formed a distinct monophyletic cluster (assigned as Linden cluster) within the genus *Rhizobium* and represent a novel *Rhizobium* species. Genomic fingerprinting by BOX- and GTG5-PCRs, and DNA-DNA hybridization indicated a high genetic variability within the cluster. Differences in carbon substrate utilization pattern revealed furthermore a high physiological diversity. The ecological role of the Linden Cluster needs to be investigated in more detail. However, differences in substrate utilization pattern so far indicated the adaptation of the different strains representing the cluster to different ecological niches within the grassland, caused by different plant species and climate change conditions. This may be explained by the fact that different plants as well as the same plant growing under different environmental conditions have specific sugar excretion patterns affecting the growth of different microbes inhabiting the phyllosphere.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

724/MDEP
***Streptococcus dysgalactiae* isolated from human and animal clinical cases – are the subspecies differentiation criteria still accurate?**
M. Ciszewski*[1], E. Szewczyk[1]
[1]*Medical University of Lodz, Department of Pharmaceutical Microbiology and Microbiological Diagnostics, Lodz, Poland*

Introduction: *Streptococcus dysgalactiae* is a new zoonotic human pathogen. Two decades ago it was considered as streptococcus pathogenic only to animals. Nowadays, isolations of this species strains from human infections is becoming more and more frequent. Those infections are clinically similar to ones caused by *Streptococcus pyogenes* and occasionally are life-threatening. In 1990s, two subspecies: *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) and *Streptococcus dysgalactiae* subsp. *dysgalactiae* (SDSD) were established. Over the years, controversies arose over this classification, showing that the taxonomy of genus *Streptococcus* has not been completed yet and *S. dysgalactiae* division into subspecies generates more questions than answers.
Objectives: The aim of this study was to identify *S. dysgalactiae* strains from recently obtained clinical samples to the subspecies level. The subspecies differentiation criteria described in the literature, as well as modern molecular methods, were employed. The presented study provides a new view on *S. dysgalactiae* identification and subspecies differentiation criteria.
Materials & methods: We examined 30 *S. dysgalactiae* isolates from human clinical samples and 6 *S. dysgalactiae* isolates from animal clinical samples from pets (dogs). These strains, initially identified as *S. dysgalactiae*, were subsequently assigned to SDSE or SDSD subspecies by means of phenotypic observations, such as: hemolytic properties, Lancefield group and the production of streptokinase able to activate human plasminogen. These results were compared with the identification according to modern molecular methods: RISA and 16S rDNA sequencing.
Results: The identification to the subspecies level according to generally accepted phenotypic criteria proved to be inconsistent and contrary to the molecular identification results. The latter

allowed to unambiguously identify all analyzed isolates as *S. dysgalactiae* subsp. *equisimilis* (SDSE).
Conclusion: The results of identification of the group of 36 isolates question the subspecies division criteria described both by Vandamme et al. and by Vieira et al. in 1990s. This study, as well as observations of inconsistencies present in the majority of publications concerning *S. dysgalactiae*, lead to the discussion about the reasons of this situation and encourage to change the taxonomy of this species. Due to the wide diversity of *S. dysgalactiae* strains we suggest to assign pathotypes, rather than subspecies. Pathotypes names should reflect the pathogenicity to a certain host. However, if the division into two subspecies remains, which in the light of the presented data might be clinically negligible, the division into subspecies should base mainly on genetic analyses by means of DNA sequences unique for the subspecies (DNA fingerprint) including genetic method described by Preziuso et al. in 2014.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

725/MDEP
Diversity and dynamics of *Staphylococcus aureus* during chronic airway infection of cystic fibrosis patients
L. Ueberhorst*[1], T. Thissen[1], B. Husmann[1], S. Deiwick[1], H. Rengbers[1], A. Dübbers[1], C. Kessler[1], J. Große-Onnebrink [1], P. Küster[1], H. Schültingkemper[1], B. Kahl[1]
[1]*Medizinische Mikrobiologie, Münster, Germany*

Background: Cystic fibrosis patients suffer from chronic recurrent bacterial airway infections, which ultimately lead to lung insufficiency and decreased life expectancy. *Staphylococcus aureus* is one of the earliest and one of the most common pathogens isolated from the airways of CF patients. The diversity of *S. aureus* during chronic airway infection is not known. Therefore, we conducted a prospective study to determine the dynamics of diverse pheno- and or genotypes in the lung habitat of patients chronically infected by *S. aureus* during a one-year period.
Methods: We selected 14 patients of 2 CF-centers in Münster, who were persistently infected by *S. aureus* and regularly expectorate sputum. From every sputum, we isolated 40 colonies and determined diversity by evaluating size (normal/SCV), hemolysis (β-hemolysis/β-toxin/non-hemolytic) on Columbia blood), mucoidy on Kongored agar (non-mucoid/mucoid), resistance profiles and *spa* types.
Results: The median age of patients was 24 years (range 16, 45). Nine patients (70%) were co-infected by *Pseudomonas aeruginosa*. Preliminary results of 52 sputa (n=2080 isolates) from 14 patients revealed a high diversity of phenotypes within individual sputa (1 -20 phenotypes, mean 5). The number of phenotypes varied during sequential visits. 396 (19%) isolates were stable or dynamic SCVs, 1542 (74%) were hemolytic, 251 (12%) were β-toxin positive; 332 (16%) isolates displayed a mucoid phenotype on Kongored agar. 226 (11%) isolates were PSSA, 1448 (70%) MSSA and 406 (20%) MRSA. Most patients were infected by a single clonal lineage.
Conclusions: Our preliminary results revealed a high diversity of *S. aureus* phenotypes during persistent airway infection, which varied within and also between patients. Associations of changing phenotypes with bacterial density, exacerbation, co-infection with *P. aeruginosa* or antibiotic therapy will be further evaluated.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

726/MDEP
High diversity of marine Actinobacteria from Easter Island, Chile
I. Sottorff*[1], J. Wiese[1], J. F. Imhoff[1]
[1]*GEOMAR Helmholtz Centre for Ocean Research Kiel, Marine Microbiology, Kiel, Germany*

Introduction: The exploration of new environments always has been an important task in terms of microbial diversity studies, ending up in the discovery of new species, interactions, and eventually economic products. Additionally, it has worked as a reference point in which it allows us to compare environments with a hard anthropogenic influence versus other with no influence at all. Moreover, the biodiversity assessment has become a relevant topic when contrasted with the biogeography of microbes to determine whether or not there is a close relationship between environment specificity and species, or on other hand a cosmopolite distribution.
Objectives: The aim of this work is to explore the diversity of marine Actinobacteria dwelling around the Easter Island, Chile. This location has been chosen, because it is the most remote island in the ocean apart from the large continents.
Materials and Methods: We took samples from the coastal waters and sediments around Easter Island and used 7 different culture media for the isolation of Actinobacteria. Each medium was supplemented with complex sugars and sea water. Axenic cultures obtained after repeated sub-cultivation were identified by phylogenetic analysis of the 16S rRNA gene sequences.
Results: A total of 96 isolates of Actinobacteria had representatives in 18 different genera, such as *Arthrobacter, Micromonospora, Salinispora, Dactylosporangium, Marmoricola, Nonomuraea, Streptomyces*, *Verrucosispora, Aeromicrobium, Geodermatophilus, Dietzia, Yimella, Rhodococcus, Nocardioides, Blastococcus, Microbacterium, Cellulosimicrobium, and Ornithinimicrobium.* The genera with the highest diversity were *Micromonospora* with 21 different genotypes and *Streptomyces* with 15 genotypes. A number of the isolates revealed low sequence similarity of 16S rRNA gene to phylogenetic neighbors according to the Blast-NCBI data base. Despite of the remote location of the island, a larger number of the species identified has been known previously from other places of the world. Ongoing studies are dealing with the characterization of the isolates and their biochemical potential, specifically their capacity of producing secondary metabolites.
Conclusion: The coastal habitats of Easter Island have proven to harbor considerable high diversity of Actinobacteria with representatives of 18 different genera isolated from a total number of 98 isolates obtained. The most abundant genera were *Micromonospora* and *Streptomyces*, representing 55.2% of all isolates. Although, the abundance of these two genera is significant, the other genera play a relevant role due to their variety. Therefore, the Easter Island coastal zone can be regarded as a rich source of diverse Actinobacteria, some of which are likely to represent new species or higher taxa.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

727/MDEP

Applying PacBio SMRT sequencing for rapid identification and differentiation of bacterial strains

S. Krahn*[1,2], C. Heiner[3], R. Hall[3], S. Oh[3], A. Vilcinskas[1,4], J. Glaeser*[1,2]
[1]*Fraunhofer IME, Bioressources, Giessen, Germany*
[2]*Sanofi-Fraunhofer, Natural Products Center, Frankfurt, Germany*
[3]*Pacific Biosciences, Menlo Park, California, United States*
[4]*Justus-Liebig University, Institute for Insect Biotechnology, Giessen, Germany*

16S rDNA sequence information is very valuable for the differentiation of bacteria at the genus level. However, due to the limited variability of the 16S rDNA sequence, the value for differentiation at lower taxonomic levels is sometimes limited (e.g. in *Streptomyces* spp.). In contrast, the 16S-23S spacer region (ITS) often has a high interspecies and intraspecies variability and thus can complement the 16S rDNA sequence information to discriminate bacterial isolates at the sub-species level. PacBio SMRT (Single Molecule, Real-Time) sequencing is a single molecule sequencing technology which allows the generation of reads with an average lager than 10,000 bp. We used the PacBio SMRT long read circular consensus sequencing technology to sequence 16S rDNA plus the 16S-23S spacer region in one run. Here we show how this approach can be used to rapidly identify and discriminate a large number of bacterial strains simultaneously. For this purpose, barcoded amplicons that cover the 16S rDNA and the 16S-23S spacer region of 96 bacterial cultures were generated and analyzed by PacBio SMRT sequencing. We show that efficient quality filtering of the sequencing reads resulted in OTUs of high accuracy and demonstrate that the sequencing information can be used for strain identification. This application allows the detection of strain duplicates and bacterial contaminations in large bacterial strain collections and in samples from high throughput isolation approaches and therefore can be used as quality control and evaluation of cultured diversity even at lower taxonomic levels at a reasonable cost.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

728/MDEP

Phylogenetic Analysis of Prevalent Tuberculosis and Non-Tuberculosis Mycobacteria in Isfahan, Iran, Based on a 360 bp Sequence of the *rpoB* Gene

B. Nasr Esfahani*[1]
[1]*Isfahan University of Medical Sciences, School of Medicine, Microbiology, Isfahan, Islamic Republic Of Iran*

Background: Taxonomic and phylogenetic studies of *Mycobacterium* species have been based around the *16sRNA* gene for many years. However, due to the high strain similarity between species in the *Mycobacterium* genus (94.3% - 100%), defining a valid phylogenetic tree is difficult; consequently, its use in estimating the boundaries between species is limited. The sequence of the *rpoB* gene makes it an appropriate gene for phylogenetic analysis, especially in bacteria with limited variation.
Objectives: In the present study, a 360bp sequence of *rpoB* was used for precise classification of *Mycobacterium* strains isolated in Isfahan, Iran.
Materials and Methods: From February to October 2013, 57 clinical and environmental isolates were collected, subcultured, and identified by phenotypic methods. After DNA extraction, a 360bp fragment was PCR-amplified and sequenced. The phylogenetic tree was constructed based on consensus sequence data, using MEGA5 software.
Results: Slow and fast-growing groups of the *Mycobacterium* strains were clearly differentiated based on the constructed tree of 56 common *Mycobacterium* isolates. Each species with a unique title in the tree was identified; in total, 13 nods with a bootstrap value of over 50% were supported. Among the slow-growing group was *Mycobacterium kansasii*, with *M. tuberculosis* in a cluster with a bootstrap value of 98% and *M. gordonae*in another cluster with a bootstrap value of 90%. In the fast-growing group, one cluster with a bootstrap value of 89% was defined, including all fast-growing members present in this study.
Conclusions: The results suggest that application of the *rpoB* gene sequence is sufficient for taxonomic categorization and definition of a new *Mycobacterium* species, due to its high resolution power and proper variation in its sequence (85% - 100%); the resulting tree has high validity.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

729/MDEP

Heterogeneous toxin production promotes success in two-strain bacterial competition

B. von Bronk[1], S. Schaffer[1], M. Opitz*[1]
[1]*LMU, Fakultät Physik, München, Germany*

Interactions like cooperation and competition between different organisms govern ecosystem dynamics. To investigate such interactions in bacterial model systems from single-cell level to mature macro-colonies, we developed a new experimental approach using a microscope with zoom functionality. In combination with computational modelling, we show that competition between a bacterial strain C that heterogeneously produces a bacterial toxin and a strain which is sensitive to this toxin (S) can lead to four different outcomes: dominance of S or of C, coexistence, and extinction of both strains. Competitive success of the C strain is only observed for intermediate toxin producer fractions, the ratio of toxin producers and reproducers within the C strain population. Furthermore, we show that alternative competitor strategies, such as resistance to the toxin or accelerated growth, considerably increased competitors success. Our findings significantly enhance our understanding of indirect cooperation by means of phenotypic heterogeneity in the context of bacterial competition.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

730/MDEP

Reconciliation of mathematical modelling using tetranucleotide usage patterns with whole bacterial genomes to solve phylogenomic problem

X. Yu*[1], O. Reva[1]
[1]*University of Pretoria, Bioinformatics, Sandton, South Africa*

Nowadays, complete genome sequences of multiple bacteria became readily available for analysis. Current work which uses whole genome based alignment (WGS) approach for phylogenetic and phylogenomic research believe to resolve contradiction between gene based trees, but this approach multiplies the problem in terms of gene annotation, orthology prediction and inadequate alignment of sequences. Therefore one of the most prospective ways for genome comparison and phylogenomic inferences is then based on annotation-and-alignment free genome linguistic approaches, i.e. comparison of oligonucleotide usage patterns (OUP) of genome-scale DNA fragments. Until now this approach still lacks a reliable evolutionary model to explain the mechanisms and dynamics of changes in OUP which hinders the application of this approach to systematically compare to other well-known methods such as marker genes and/or whole genome sequence based alignment. Due to the shortfalls of individual phylogenomic methods, the lack of biological information is believed to cause phylogenomic inferencing problems. The aim of the current work is divided into three important topics: i)

comparative analysis of multiple complete genome sequences representing different phylogenetic branches at different taxomic levels to identify the driving forces of OUP evolution; ii) Analysis of topological incongruences between whole genome alignments and alignment free OUP patterns; iii) Improving phylogenetic inference by reconciliation of sequence based and pattern based evolutionary models. The major output of this research is an innovative evolutionary model implemented in a form of a computer program for phylogenetic inferences based on combination of alignment based and alignment free approaches.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

731/MDEP

Intraclonal fitness of isolates of the major *Pseudomonas aeruginosa* clones and isolates from CF patients

S. Fischer*[1], N. Cramer[1], J. Klockgether[1], S. Hedtfeld[1], M. Dorda[1], L. Wiehlmann[1], B. Tümmler[1]
[1]*Medizinische Hochschule Hannover, Hannover, Germany*

Pseudomonas aeruginosa is a common Gram-negative bacterium that can cause disease in plants and humans. The organism is considered opportunistic in so far as serious infection is often superimposed upon acute or chronic morbidity – most notably cystic fibrosis and traumatic burns – or found in immunocompromised individuals.
In this work we grew serial isolates of 12 CF patient courses (6 mild and 6 severe courses) as well as isolates of the major *P. aeruginosa* clones C and PA14 (environmental, acute and chronic infections isolates) competitively in nutrient rich LB-medium and in nutrient poor minimal medium respectively. At specific timepoints (48 h after starting the experiment with medium change every 12 h (48 h time point), followed by 72 h incubation without medium change (120 h time point)) samples were taken and sequenced and the percentage of each isolate within the experiment was calculated based on isolate specific SNPs.
In patients with a severe course there was a trend for the first isolate outcompeting all other isolates. On the other side, one of the last isolates outcompeted all others in most cases of patients with a mild clinical course.
In contrast to the finding in the serial isolates we were not able to identify single isolates that outcompeted all others in the competition of the C and PA14 isolates. Winners were identified by a gain of biomass compared to the start of the experiment. To our surprise the winners in nutrient rich medium - which should mimic the CF lung - were isolates from the environment and in minimal medium isolates from CF lungs. Screening the specific mutations of the isolates we were not able to identify any single mutation that was obviously responsible for fitness advantages, and thus we concluded that combinations of mutations are responsible for the observed competitive fitness.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

732/MDEP

***Rodentibacter* gen. nov. including *Rodentibacter pneumotropicus* comb. nov., *Rodentibacter heylii* sp. nov. and 5 more species and 2 genomospecies**

H. Christensen*[1], S. Adhikary[1], W. Nicklas[2], M. Bisgaard[1], R. Boot[1,3], P. Kuhnert[4], T. Waberschek[2], B. Aalbæk[2], B. Korczak[2]
[1]*University of Copenhagen, Veterinary Disease Biology, Frederiksberg, Denmark*
[2]*German Cancer Research Centre, Heidelberg, Germany*
[3]*emeritus, Utrecht, Netherlands*
[4]*University of Bern, Bern, Switzerland*

A new genus of *Pasteurellaceae* is proposed as *Rodentibacter* with the type species *Rodentibacter pneumotropicus*, reclassified from [*Pasteurella*] *pneumotropica.* The genus was proposed based on isolation and phenotypic characterization of more than 1000 strains, predominantly from rodents. A subset of strains was compared by partial *rpoB* sequencing and the strains showed 86 % or higher *rpoB* similarity which indicated a genus level relationship within *Pasteurellaceae*. When the strains were compared at 16S rRNA gene sequence level they showed 93.8 % or higher similarity and the genus level relationship within *Pasteurellaceae* was also confirmed by phenotypic analysis. Whole genomic comparison of selected strains allowed the estimation of DNA-DNA renaturation and indicated the presence of 10 species within the genus. The type species is *R. pneumotropicus* including strains mainly from mice. *Rodentibacter heylii* was proposed for a group that included the biovar Heyl of [*Pasteurella*] *pneumotropica* and also included predominantly strains from mice. *Rodentibacter ratti* which included the taxon 22 of Bisgaard was proposed for a new species mainly isolated from rats. Two more species were also proposed that exclusively included strains from rat, *Rodentibacter heidelbergensis* and *Rodentibacter trehalosifermentans*. Three strains including taxon 41 of Bisgaard were proposed as *Rodentibacter myodis*. Two strains including the reference strain of taxon 17 of Bisgaard were proposed as *R. rarus* and another group with seven strains were proposed as the new species *Rodentibacter mrazii*. The eight species could be separated by phenotypic characteristics. Two genomospecies could not be separated by phenotype from the 8 species outlined above and they were left unnamed. The taxonomic changes will improve the identification of members of *Pasteurellaceae* isolated from rodents since it has been known for a long time that [*Pasteurella*] *pneumotropica* is not a member of *Pasteurella sensu stricto* and that biovars Jawets and Heyl of [*Pasteurella*] *pneumotropica* should be classified as separate species. In addition a range of unnamed taxa within the [*Pasteurella*] *pneumotropica* complex have now been proposed as new species and they will allow proper indexing in the relevant databases.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

733/MDEP

***Streptococcus tigurinus* is frequent among *gtfR*-negative *S. oralis* isolates and in the human oral cavity but highly virulent strains are uncommon**

G. Conrads*[1], S. Barth[1], M. Möckel[1], L. Lenz[1], M. van der Linden[2], K. Henne*[1]
[1]*University Hospital RWTH, Division of Oral Microbiology and Immunology, Aachen, Germany*
[2]*University Hospital RWTH , Institute of Medical Microbiology and National Reference Center for Streptococci, Aachen, Germany*

Question: *Streptococcus tigurinus* is a new member of the streptococcal mitis-group and associated with infective endocarditis. Among the reference strains, *Streptococcus oralis* ATCC 35037^T, ATCC 49296, and ATCC 6249 (formerly *S. mitis*), are the nearest relatives to *S. tigurinus.* Low and high virulent variants have been described.

Methods: We searched in our national reference collection of endocarditis isolates for *S. tigurinus*-like strains sequencing housekeeping genes (16S rRNA-gene, *gdh*, *groEL*, *sodA*). The strains were further profiled by PCRs targeting virulence genes (*rib*-like, *cshA*-like, *gtfR*, *int*, *pitA, hylA*). To study the prevalence and abundance of *S. tigurinus* in saliva and on mucosal-membranes of 35 healthy adults, PCRs detecting two variants of the 16S operon and virulence genes were applied.
Results: Among our endocarditis isolates we found eight strains (all *gtfR*-negative and former *S. oralis*) holding the specific *S. tigurinus* 16S-motif but the pattern of genes related to high virulence found in the *S. tigurinus* type strain could not be detected in any of these strains. A close phylogenetic proximity between *S. tigurinus* and *S. oralis* was observed with intersectional hybrid strains formed. This was supported by concatenated housekeeping sequences, *in silico* DNA-DNA hybridization, pathogenomic profiling, and multidimensional scaling. In the oral samples, *S. tigurinus* could be detected frequently, especially in the most common operon variant, but none of the type strain related virulence factors were found.
Conclusions: Low virulent *S. tigurinus*-like strains can be found frequently and in high prevalence (66%) and abundance (12.5%) in the oral cavity of healthy adults. In strain collections (including blood isolates), they are among the formerly known *gtfR*-negative *S. oralis*. Highly virulent strains seem to be uncommon. Though closely related, *S. oralis* and *S. tigurinus* can be separated by the presence or absence of *gtfR* and dextran production. Hybrids of both species can be found. The variable arsenal of virulence genes found in our study emphasizes the genetic plasticity of mitis group streptococci. There are several parallels between the triplets *S. oralis* - *S. tigurinus* (low virulent variant) - *S. tigurinus* (high virulent variant) and *S. mitis* - *S. pseudopneumoniae* - *S. pneumoniae*.

Figure 1

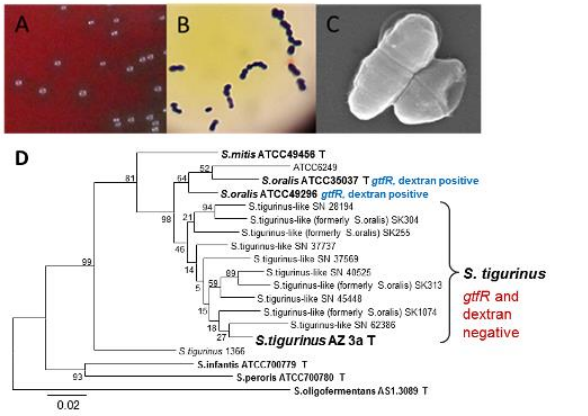


Figure Abstract 782: Represented are the smooth but dextran-negative, circular, convex and white to grey colonies of *S. tigurinus* Az_3a^T (A), a light microscopic picture after Gram stain (B), cells under the electron microscope (C), and a phylogenetic tree based on 16S-sodA-gdh-groEL concatemers (D).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

734/MDEP
Systematics: Towards an organism based system
B. J. Tindall*[1]
[1]*Leibniz Institute DSMZ, , Braunschweig, Germany*

Systematics is a fundamental aspect in the biological sciences that can be succinctly described as the "cradle of comparative biology". It has as its basis elements of theory and philosophy as well as practical experimental aspects. In recent decades systematics has suffered because it has become equated with the perception that there are immense numbers of unnamed taxa all of which have to be named as quickly as possible based on the smallest data set possible. There are fundamental problems with this approach, one of which is the impression that systematics is simply nomenclatural stamp collecting. Current trends appear to be moving towards a minimalistic, reductionist approach that relies heavily on the principle of overall similarity and cut-off values that largely ignore the biology of the organism being studied. There are elements in the current trends that reflect debates that arise at regular intervals in this discipline and for those familiar with the historical aspects of systematics recognise as a "déjà vu" effect. In essence systematics is again at a major crossroads where different schools of thought are splitting it into two or more seemingly incompatible directions. It would be prudent to examine lessons learnt from the past and to ask whether systematics is to be uncoupled from one of its fundamental aspects as serving as the "cradle of comparative biology", in essence ignoring the biology of the organisms that it deals with.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

735/MDEP
Evolution of host-microbe interactions: *Curvibacter* sp., the dominant *Hydra* colonizer
T. Wein*[1], C. Kreutzer[1], T. Dagan[1], T. Bosch[1], S. Fraune[2], N. Hülter[1]
[1]*Christian-Albrechts University of Kiel, Institute of Microbiology, Kiel, Germany*
[2]*Christian-Albrechts University of Kiel, Institute of Zoology, Kiel, Germany*

Microbial species are often found in close symbiosis with various plant and animal hosts. The β-proteobacterium *Curvibacter sp.* is the main colonizer of the freshwater polyp *Hydra vulgaris*. The *Hydra-Curvibacter* interaction is highly specific, yet coevolution and genetic mechanisms underlying colonization and symbiosis formation remain elusive. In this study, genomes of different *Curvibacter* sp. strains isolated from distinct *Hydra* hosts were analysed regarding their phylogenetic relationships. Adaptive evolution of mutualistic *Curvibacter* sp. strains was investigated by comparative genomics identifying candidate genes under positive selection. To study microbial genetics that are essential for the symbiosis we established *Curvibacter* sp. as a model system in the laboratory. An RSF1010-derived broad-host-range plasmid encoding different fluorescent proteins was constructed within an *E. coli* host and could be effectively transferred into *Curvibacter* sp. via conjugation. Transconjugants appeared already after two hours with a transfer frequency of 5x10-5 transconjugants per recipient cell. No fitness cost was found to be associated with the plasmid carriage and expression of fluorescent proteins. Fluorescently labelled *Curvibacter* sp. cells were observed and visualized in colonization experiments on *H. vulgaris*. To study the colonization process in greater detail, a gene targeting system for the generation of chromosomal knockout mutations of identified candidate genes in *Curvibacter* sp. was developed. Our approach constitutes a general framework for future research of *Hydra-Curvibacter* interaction and supplies the first glimpse into *Hydra* colonization dynamics *in vivo* in real time.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

736/MDEP

Acquisition of the capsule locus by horizontal gene transfer in *Neisseria meningitidis* is often accompanied by the loss of UDP-GalNAc synthesis

S. N. Bartley[1,2], S. Mowlaboccus[1], C. A. Mullally[1], K. A. Stubbs[2], A. Vrielink[2], M. C. J. Maiden[3], O. B. Harrison[3], T. T. Perkins[1], C. M. Kahler*[1]
[1]*University of Western Australia, School of Pathology and Laboratory Medicine, and the Marshall Centre for Infectious Disease Research and Training, Perth, Australia*
[2]*University of Western Australia, School of Chemistry and Biochemistry, Perth, Australia*
[3]*University of Oxford, Department of Zoology, Oxford, United Kingdom*

Pathogenic meningococci have acquired a 24 kb capsule synthesis island (*cps*) by horizontal gene transfer which consists of a synthetic locus and associated capsule transport genes flanked by repetitive Regions D and D". Regions D and D" contain an intact gene encoding a UDP-galactose epimerase (*galE1*) and a truncated remnant (*galE2*), respectively. In this study, GalE protein alleles were shown to be either mono-functional, synthesising UDP-galactose (UDP-Gal), or bi-functional, synthesising UDP-Gal and UDP-galactosamine (UDP-GalNAc). Meningococci possessing a capsule null locus (*cnl*) typically possessed a single bi-functional *galE*. Separation of functionality between *galE1* and *galE2* alleles in meningococcal isolates was retained for all serogroups except serogroup E which has a synthetic requirement for UDP-GalNAc. The truncated *galE2* remnant in Region D" was also phylogenetically related to the bi-functional *galE* of the *cnl* locus suggesting common ancestry. A model is proposed in which the illegitimate recombination of the *cps* island into the *galE* allele of the *cnl* locus results in the formation of Region D" containing the truncated *galE2* locus and the capture of the *cps* island en bloc. The retention of the duplicated Regions D and D" enables inversion of the synthetic locus within the *cps* island during bacterial growth.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Microbial Pathogenesis (FG MP)

737/MPP

Analysis of gene expression in *Acinetobacter baumannii* under stress conditions using reporter gene assays

S. Zeidler*[1], J. Hubloher[1], A. L. Siemund[1], V. Müller[1], B. Averhoff[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt / Main, Germany*

Introduction: *Acinetobacter baumannii* is an opportunistic human pathogen emerging in intensive care units in hospitals worldwide [1, 2] with recent outbreaks also in Germany. Besides the existence of multidrug resistant strains, *A. baumannii* has the extraordinary capacity to survive in dry environments, which enables the bacterium to persist in the hospital environment [3]. Generally speaking, *A. baumannii* is a very versatile organism able to cope with various stresses and to adapt efficiently to adverse and changing environmental conditions.
Objectives: Reporter gene assays were used to get insight into gene expression of *A. baumannii* under different stress conditions. The proteins of interest were the three phospholipases D, which are known to contribute to virulence [4], and enzymes involved in synthesis of compatible solutes and therefore important for osmotic stress resistance, namely the mannitol-1-phosphate dehydrogenase/phosphatase (MtlD) as well as the trehalose-6-phosphate-synthase and -phosphatase (OtsA and OtsB).
Materials & Methods: The putative promoter regions of the osmotic stress related genes *mtlD* and *otsAB* from *A. baumannii* ATCC 19606 were fused to the reporter gene *gusA*, encoding a β-glucuronidase. *A. baumannii* cells were transformed with the plasmid and the promoter activity was investigated *in trans*. Gene expression of the phospholipases D was analyzed *in cis* using *lacZ* fusions, so that the β-galactosidase served as a reporter for promoter activity.
Results: Expression of the phospholipases D is controlled by temperature and growth phase. Production of the compatible solutes mannitol and trehalose in *A. baumannii* is regulated on the transcriptional level. The presence of NaCl and other osmotic active substances stimulates transcription of *mtlD* and *otsAB*.
Conclusion: Reporter gene assays were established as a useful tool to elucidate gene expression of *A. baumannii* ATCC 19606 in response to various stress factors.

References

[1] L. Dijkshoorn, A. Nemec, and H. Seifert. *Nat. Rev. Microbiol.* 5 (2007), 939-951.
[2] A. Howard, M. O'Donoghue, A. Feeney, and R. D. Sleator. *Virulence* 3 (2012), 243-250.
[3] A. Jawad, H. Seifert, A. M. Snelling, J. Heritage, and P. M. Hawkey. *J. Clin. Microbiol.* 36 (1998), 1938-1941.
[4] J. Stahl, H. Bergmann, S. Göttig, I. Ebersberger, and B. Averhoff. *Plos One* 10 (2015).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

738/MPP

The role of phospholipase A PlbF of *Pseudomonas aeruginosa* for virulence

C. H. Strunk*[1], M. Caliskan[1], F. Bleffert[1], J. Granzin[2], M. Siebers[3], P. Dörmann[3], L. Rahme[4], R. Batra-Safferling[2], K. E. Jaeger[1,5], F. Kovacic[1]
[1]*Heinrich-Heine-University Düsseldorf, Institute of Molecular Enzymtechnology, Jülich, Germany*
[2]*Forschungszentrum Jülich GmbH, Institute of Complex Systems ICS-6: Structural Biochemistry, Jülich, Germany*
[3]*University of Bonn, Institute of Molecular Biotechnology IMBIO, Bonn, Germany*
[4]*Harvard Medical School, Department of Microbiology and Immunology, Boston, Massachusetts, United States*
[5]*Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences IBG-1: Biotechnology, Jülich, Germany*

The Gram-negative human pathogen *Pseudomonas aeruginosa* causes a wide range of infections with severe morbidity and mortality cases1. Among many virulence factors produced by *P. aeruginosa* are several phospholipases generally known to contribute to phospholipid homeostasis, damage of host cell membranes and modulation of lipid signaling in eukaryotic cells2,3. Recently, fatty acid derivatives called diffusible signal factors (DSF) were related to virulence of several bacterial pathogens; however, DSF biosynthesis and signaling pathways are largely unknown4.
Here, we report on PlbF, a newly identified phospholipase A of *P. aeruginosa* which hydrolyses various phospholipids *in vitro* and *in vivo* releasing medium chain fatty acids. PlbF is anchored to the cytoplasmic membrane *via* a single transmembrane (TM) helix and exists in monomeric and dimeric forms. Biochemical and structural data suggesting regulation of PlbF function through reversible dimerization triggered by fatty acids. Presently, we study dynamics of PlbF dimerization in phospholipid vesicles which should reveal the role of specific phospholipids for PlbF function.
A *P. aeruginosa* Δ*plbF* deletion strain is affected in biofilm formation and showed reduced virulence in a *Drosophila melanogaster* infection model pointing to PlbF as a novel virulence factor of *P. aeruginosa*. Since PlbF is not in direct contact with host cells it is intriguing how PlbF influences virulence of *P. aeruginosa*. To identify other proteins involved in PlbF-mediated virulence we have performed pull down

experiments, which revealed the cell division protein FtsZ and flotillin-like protein Flo2 as putative interaction partners of PlbF. FtsZ presumably mediates invagination of cytoplasmic membrane during the cell division, whereby Flo2 shows homology to eukaryotic proteins related to membrane fusion during endocytic processes. We propose the role of PlbF in cytoplasmic membrane remodelling processes as response to increased concentration of fatty acids or DSF molecules.

References

[1] Gellatly, S. L., *et al.* (2013) *Dis.* 67, 159-173
[2] Zhang, Y. M., *et al.* (2008) *Rev. Microbiol.* 6, 222-233
[3] Sitkiewicz, I., *et al.* (2007) *Trends Microbiol.* 15, 63-69
[4] Ryan, R. P. & Dow, J. M. (2011) Trends Microbiol 19, 145-152.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

739/MPP

Glycosylated plasmin-sensitive protein Pls in methicillin-resistant *Staphylococcus aureus* (MRSA) promotes biofilm formation

J. Eikmeier[1], I. Bleiziffer[1], G. Pohlentz[2], K. McAulay[3], G. Xia[4], M. Hussain[2], A. Peschel[5], S. Foster[3], G. Peters[2], C. Heilmann*[1]
[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*
[2]*University of Münster, Institute for Hygiene, Münster, Germany*
[3]*University of Sheffield, Department of Molecular Biology and Biotechnology, Sheffield, United Kingdom*
[4]*University of Manchester, Division of Infection, Immunity and Respiratory Medicine, Manchester, United Kingdom*
[5]*University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany*

Question: Until recently, the inability of bacteria to glycosylate proteins has been considered a dogma. Now it is widely accepted that bacteria can glycosylate proteins and most bacterial glycoproteins identified to date are virulence factors of pathogenic bacteria, i.e. adhesins and invasins. However, the impact of protein glycosylation on the major human pathogen *Staphylococcus aureus* remains incompletely understood. Therefore, we aimed to identify *S. aureus* surface glycoproteins, analyze the underlying glycosylation machinery and characterize the function of the glycosyl modifications.
Methods: To study protein glycosylation in staphylococci, we analyzed lysostaphin lysates of methicillin-resistant *Staphylococcus aureus* (MRSA) strains by SDS-PAGE, subsequent periodic acid-Schiffs staining, and mass spectrometry.
Results: We found that the plasmin-sensitive surface protein Pls is a post-translationally modified glycoprotein. *pls* is encoded by the SCC*mec* type I in MRSA and has been demonstrated to be a virulence factor in mouse septic arthritis. In a search for glycosyltransferases, we identified two open reading frames encoded downstream of *pls* on the SCC*mec* element, which we termed *gtfC* and *gtfD*. Expression and deletion analysis revealed that both *gtfC* and *gtfD* mediate glycosylation of Pls. Additionally, the recently reported glycosyltransferases SdgA and SdgB are involved in Pls glycosylation. Glycosylation occurs at serine residues in the Pls SD-repeat region and modifying carbohydrates are *N*-acetylhexosaminyl residues. Functional characterization revealed that Pls can confer increased biofilm formation, which seems to involve two distinct mechanisms. The first mechanism depends on glycosylation of the SD-repeat region by GtfC/GtfD and probably also involves eDNA, while the second seems to be independent of glycosylation as well as eDNA. Other previously known Pls properties are not related to the sugar modifications.
Conclusions: In conclusion, Pls is a glycoprotein and Pls glycosyl residues can stimulate biofilm formation. Thus, sugar modifications may represent promising new targets for novel therapeutic or prophylactic measures against life-threatening *S. aureus* infections.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

740/MPP

Characterization of the novel cysteine protease EspL of enteropathogenic *E. coli*

A. K. Riebisch*[1], P. Dersch[1], S. Mühlen[1]
[1]*Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany*

Enteropathogenic *Escherichia coli* (EPEC) is a gastrointestinal pathogen that causes diarrheal disease, especially in infants. EPEC adheres to the mucosomal surface of enterocytes where it forms a pedestal structure, like pseudopodia, to support dissemination and multiplication. Like enterohemorrhagic *E. coli* (EHEC), EPEC injects a variety of effector proteins into the host cell by a type III secretion system (T3SS) to promote its survival, which is crucial for infection establishment. The genes required for the formation of the T3SS as well as genes encoding a subset of effector proteins are located either within the locus of enterocyte effacement (LEE) pathogenicity island or outside the LEE (non-LEE encoded [Nle] effectors).
Many Nle effectors are playing an important role in interfering with host defense processes such as NF-kB and MAPK signaling, apoptosis and necroptosis. Adapter proteins such as RIP Kinase 1 (RIPK1) and RIP Kinase 3 (RIPK3) that contain receptor-interacting protein (RIP) homotypic interaction motif (RHIM) are playing an important role in inflammatory signaling, especially in necroptosis. EPEC uses its T3SS effector EspL to degrade RHIM containing proteins such as RIPK1 and RIPK3 during infection. The cleavage within the RHIM domains requires a cysteine protease motif in EspL (Cys47, His131, Asp153) and leads to an inhibition of inflammatory, inflammasome and necroptotic signaling. Due to this role in disrupting these protective immune response, EspL is an important virulence factor for EPEC.
Apart from the cysteine protease motif of EspL, little is known about other functional sites of the enzyme. Here, we will use random transposon mutagenesis to identify possible interaction sites and amino acid residues that are required for the virulence function of EspL.
Following experiments such as transfection, immunoprecipitation and reporter-gene assays will then allow to determine amino acids of EspL that are required for its virulence function.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

741/MPP

Mechanisms of the adaption and persistence of *Yersinia enterocolitica*

C. Schmühl*[1], A. Nuss[1], A. K. Heroven[1], M. Beckstette[1], P. Dersch[1]
[1]*Helmholtz-Centre for Infection Research, Molecular Infectionbiology (MIBI), Braunschweig, Germany*

Gastrointestinal diseases are the cause of approximately 2.5 million deaths per year and are therefore a huge problem concerning public health in all parts of the world. *Y. enterocolitica* is one major cause of gastrointestinal infections. Pigs are considered as a natural reservoir host for *Y. enterocolitica*, making raw or undercooked pork meat one of the main sources of infections for humans. Once inside the human body, these bacteria cause several symptoms associated with the gastrointestinal tract like diarrhea, enterocolitis or abdominal pain. Moreover, *Y. enterocolitica* is able to establish systemic infections causing reactive arthritis or mesenteric lymphadenitis. These diseases caused by enteropathogenic *Yersinia* are referred to as yersiniosis.
The different isolates of *Y. enterocolitica* are divided into biotypes and serotypes based on their biochemical and immune reactive

properties. Diverse serotypes are able to cause clinical manifestations in humans. Most cases of yersiniosis in Germany are caused by bioserotype 4/O:3, which is frequently isolated from humans and pigs. Bioserotyp 1B/O:8 is less prevalent, although it shows a higher virulence in mice and is therefore very well characterized in the laboratory.
The aim of this study is to investigate the differences between those two strains. In preliminary studies, it was shown that serotype O:3 is better adapted to the porcine host and shows altered invasion and adhesion properties in cell lines. Currently, the underlying mechanisms of the phenotype observed *in vivo* are further investigated *in vitro*. One approach for investigation is RNA sequencing to gain insights into the RNA expression pattern under various conditions. The comparative RNA-seq based approach allowed us to identify and compare the transcriptional start sites (TSS) of the two strains and to analyze and reveal the similarities and differences in the gene expression profile.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

742/MPP
Molecular analysis of regulatory factors and environmental signals controlling secretion systems important for *Yersinia* virulence
V. Knittel*[1], S. Seekircher[1], A. K. Heroven[1], P. Dersch[1]
[1]*Helmholtz-Centre for Infection Research, Molecular Infectionbiology (MIBI), Braunschweig, Germany*

Yersinia pseudotuberculosis is an enteric pathogen that causes gut-associated diseases in humans. The carbon storage regulator system, consisting of the RNA-binding protein CsrA and the small non-coding RNAs CsrB and CsrC, plays a major role in virulence regulation of *Y. pseudotuberculosis*. Recently, we discovered that CsrA regulates Type VI secretion system 4 (T6SS4) expression. In the absence of *csrA*, transcription of the T6SS4 operon is strongly upregulated. Additionally, we could demonstrate that this CsrA-mediated regulation occurs via a CsrA controlled and so far uncharacterized hypothetical protein. This protein was named RovC and is conserved in *Y. pseudotuberculosis* and *Y. pestis* and does not exhibit any homology to known proteins or protein domains. It is maximally expressed at 25 °C in stationary growth phase. On the one hand, CsrA was shown to repress *rovC* transcription. On the other hand, CsrA regulates RovC expression post-transcriptionally by directly binding and stabilizing the *rovC* mRNA transcript.
Expression of a translational T6SS4-*lacZ* reporter fusion is strongly upregulated upon RovC overexpression. In a *rovC* mutant strain all genes belonging to the T6SS4 operon are downregulated. DNA binding studies showed a direct interaction of RovC with the promoter region of the T6SS4 operon. The binding region was narrowed down by footprinting to a region in close proximity to the -35 and -10 region of the T6SS4 promoter. Furthermore, RovC overexpression in *Y. pseudotuberculosis* YPIII resulted in translocation of the T6SS4 effector Hcp.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

743/MPP
Toll-like receptor 2 activation depends on lipopeptide shedding by bacterial surfactants
D. Hanzelmann*[1], H. S. Joo[2], M. Franz-Wachtel[1], T. Hertlein[3], S. Stevanovic[1], B. Macek[1], C. Wolz[1], F. Götz[1], M. Otto[2], D. Kretschmer[1], A. Peschel[1]
[1]*Universität Tübingen, Tübingen, Germany*
[2]*National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, United States*
[3]*Universität Würzburg, Würzburg, Germany*

Staphylococcus aureus is a major human pathogen. USA300, a community acquired MRSA (CA-MRSA) strain, is responsible for several infection outbreaks. The master regulator of virulence, Agr, is highly active in CA-MRSA strains resulting in expression of several toxins. Among toxins phenol-soluble modulins play a crucial role in inflammation and have diverse impact on pathogenicity. Bacteria-derived lipopeptides induce a strong inflammatory immune response via Toll-like receptor 2 (TLR2). These lipopeptides are membrane-anchored proteins and are partially released by bacteria, yet it is still unclear how. We observed that *S. aureus* clinical isolates differ substantially in the capacity to activate TLR2. Therefore, we wanted to know if the heterogeneity among *S. aureus* strains in TLR2 activation correlates with the *agr* activity and PSM secretion. Here we show that PSM α2 and α3 are responsible for lipopeptide release from the cytoplasmic membrane and thereby influence TLR2 activation and inflammation. Hence, TLR2 plays a vital role particularly in these highly pathogenic *S. aureus* strains, because of the secretion of high amounts of α-PSMs. TLR2 is crucial not only for inflammation but also for protection of the host. Thus, it is important to know under which conditions TLR2 modulation might be important to treat an infection.

References
Nat Commun. 2016 Jul 29;7:12304. doi: 10.1038/ncomms12304.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

744/MPP
Characterization of the HD domain protein in *Streptococcus pyogenes*
G. Barkowsky[1], M. Schäfer*[1], N. Dehn[1], B. Kreikemeyer[1], N. Patenge[1]
[1]*Universitätsmedizin Rostock, Rostock, Germany*

Introduction: *Streptococcus pyogenes* is a Gram-positive, aerotolerant and beta-hemolytic bacterium, which causes local skin and throat infections. Additionally, it triggers generalized and toxin-mediated syndromes or long term effects such as acute rheumatic fever and glomerulonephritis. In 2014, *S. pyogenes* was responsible for 500.000 deaths. Therefore, characterization of the virulence potential and function of proteins such as the HD protein are very important. Proteins with HD domains belong to the super family of metal-dependent phosphatases and were predicted to have an impact on the nucleic acid metabolism and signal transduction. Since previous studies implied an interaction between the sRNA Moses10 and the HD-protein, the aim of this study was the purification and enzymatic characterization of the HD-protein.
Materials and Methods: *Escherichia coli* BL21 was transformed with a plasmid containing the gene for the HD-protein fused to a Strep-tag®. Induction of gene expression was followed by affinity chromatography with Strep-Tactin® Sepharose® and identification of the HD-protein with SDS-Page and Western blot analyses. Furthermore, a nuclease assay and a two-step colorimetric phosphatase assay were performed. HD-protein gene and MOSES10 gene deletion strains were constructed by homologous recombination in *S. pyogenes*.

Results: The HD-protein had an apparent molecular weight of 50 kDa and could be purified with a yield of 1.8 mg/l. Nuclease and phosphatase assays were performed using different cations and substrates. Optimal conditions for the nuclease activity were 37 °C, 4 mM MnCl2, 150 mM NaCl and pH 7.5. ssDNA and linear dsDNA were the preferred substrates. Additionally, the HD-protein exhibited phosphohydrolase activity. Optimal conditions were 37 °C, 1 mM MnCl2 and pH 8. TTP, dATP and dTTP could be identified as suitable substrates.
Conclusion: In this study, we could observe a nuclease and phosphatase activity of the HD-protein. These activities might have an influence on virulence of *S. pyogenes*. For that reason, blood survival assays will be performed with *S. pyogenes* ΔMOSES10 and ΔHD. Additionally, we will study the localization of the HD-protein in *S. pyogenes*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

745/MPP
Interplay between chemotaxis protein CheM and SPI-4 components modulates function of the SPI-4 encoded adhesin SiiE
S. Hoffmann*[1], C. Schmidt[1], R. Gerlach[1]
[1]*Robert Koch-Institute, Wernigerode, Germany*

Question: During the infection process *Salmonella enterica* has to overcome the intestinal barrier formed by polarized epithelial cells. For that functional cooperation between the SPI-1 encoded type three secretion system and the type one secretion system (T1SS) encoded on SPI-4 is required. The SPI-4 T1SS secretes the giant non-fimbrial adhesin SiiE, which mediates intimate contact of *Salmonella* to microvilli on the apical membrane of the host cell. SiiE has to be retained on the bacterial surface to allow proper adhesion and subsequent invasion into polarized cells. Previously we demonstrated that the SPI-4 encoded SiiAB form a MotAB-like proton channel which is essential for this process. Given this similarity we asked whether the chemotaxis system is involved in controlling the switch between secretion and retention of the adhesin SiiE.
Methods: Several mutants lacking up to 7 methyl-accepting chemotaxis proteins (MCP) were constructed using scarless mutagenesis and tested for invasion in polarized and non-polarized epithelial cell infection models. Possible interactions of SPI-4 components SiiA, SiiB, SiiD and SiiF with CheM were investigated in a Bacterial-Two-Hybrid system (BacTH). The ability to retain SiiE on its surface was tested in the absence and presence of α-methyl-D, L-aspartate (MeAsp), a non-metabolizable chemoattractant sensed by CheM, using the SIMPLE (screening with immunomagnetic particles for ligand expression) assay. The amounts of secreted SiiE were quantified under the same conditions with a SiiE-specific ELISA. The capability to invade polarized (MDCK) epithelial cells was assessed in the presence of MeAsp.
Results: CheM mutants showed attenuation for invasion of polarized epithelial cells comparable to a *cheY* mutant strain. In BacTH direct interactions of CheM with itself and the SPI-4 components SiiA, SiiB, SiiD, but not SiiF, were detected. Using the SIMPLE-assay we observed increased surface retention of SiiE in the presence of MeAsp. Correspondingly, the amounts of secreted SiiE were decreased in the presence of the chemoattractant. Stimulation of CheM signaling resulted also in increased invasion of polarized MDCK cells.
Conclusion: The direct interaction of CheM with SPI-4 components and modulation of SiiE surface expression by the CheM ligand MeAsp reveals a surprising functional link between a chemotaxis component and SPI-4 mediated adhesion. Our findings contribute not only to the understanding how host cells can be sensed by *Salmonella* but also how virulence functions can be controlled in a precise spatio-temporal fashion.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

746/MPP
Factors affecting optimal growth of *Xanthomonas campesteris* pv. *vesicatoria* in plant apoplast.
T. Mahto*[1], K. Matern[1], G. Sawers[1]
[1]*Martin Luther University Halle-Wittenberg, Institute of Microbiology, Halle (Saale), Germany*

Introduction: *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) is an obligately aerobic, oxidase-negative phytopathogenic *γ*-proteobacterium that causes bacterial spot disease on pepper (*Capsicum annuum*) and tomato (*Solanum lycopersicum*) plants. This pathogenic bacterium proliferates in the intercellular space (the apoplast) between plant cells. As it invades the plant tissue it encounters a variety of plant-defence responses such as oxygen, Iron and nutrient limitations and thus faces challenges for survival.
Objective: The aim this project is to investigate the influence of numerous cytochrome oxidases, as well as citrate metabolism on *Xcv* in the apoplast.
Material and methods: Various combinations of deletion mutations were introduced into Xcv using triparental conjugation. Growth studies of the resulting mutants were performed comparing them to that of wild-type *in vitro* as well as *in planta*.
Result and Conclusions: *Xcv* encodes three aconitases AcnA, AcnA2 and AcnB, which interconvert citrate and isocitrate in the citric acid cycle. *acnB* mutants exhibit reduced growth in the plant apoplast, perhaps suggesting that citrate is an important carbon substrate in this environment. *acnB* is co-transcribed with two small genes termed *roaX* and *roaY* (regulators of aconitases) and mutants lacking RoaXY shows strong up-regulation of *acnB*. In contrast, an *acnA* mutant exhibits normal growth. AcnA2 appears to have a regulatory function, controlling the synthesis of AcnA and AcnB, possibly in response to varying O2 levels. Mutants deficient in both AcnA enzymes or in AcnB plus AcnA2 are highly sensitive to ROS. Together these data suggest that these FeS proteins form an O2-responsive network that helps optimize growth of *Xcv in planta* under varying O2 concentrations.
Also, the genome of *Xcv* encodes a protein we term FLP, which is a FNR-like protein, a redox-sensitive iron- sulfur cluster (FeS)-containing transcription factors that sense oxygen and ROS directly. Bioinformatic and transcriptomic studies identified a minimal FLP-regulon in *Xcv*, whereby principally the *cydABX* operon, which encodes a high affinity cytochrome (Cyt) *d* oxidase, is positively regulated by the transcription factor. Quantitative RT-PCR experiments confirmed that in a *flp* mutant expression of the *cydABX* operon was reduced, but not abolished, indicating another as yet unidentified regulator is involved in controlling the expression of the operon. Further analysis revealed that *Xcv* encodes five oxidases namely Cyt *o*, Cyt *c*, Cyt *d*, Cyt *dII* and a cyanide-insensitive oxidase. It appears that all these together help *Xcv* to overcome oxygen limited environment condition in the plant apoplast and the absence of one or more oxidase is compensated by over-production of the remaining enzymes.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

747/MPP

Screening for protein-protein-interactions using a *Staphylococcus aureus* specific protein microarray

P. Kloppot*[1,2], M. Fuchs[1,2], E. Müller[3], R. Ehricht[3], A. Westphal[4], P. Zipfel[4], M. Hecker[5], S. Engelmann[1,2]
[1]*Technische Universität Braunschweig, Institut für Mikrobiologie, Braunschweig, Germany*
[2]*Helmholtz-Zentrum für Infektionsforschung, Mikrobielle Proteomik, Braunschweig, Germany*
[3]*Alere Technologies GmbH, Jena, Germany*
[4]*Hans-Knöll-Institut Jena, Infektionsbiologie, Jena, Germany*
[5]*Ernst-Moritz-Arndt-Universität Greifswald, Institut für Mikrobiologie, Greifswald, Germany*

The *Staphylococcus aureus* specific protein microarray *Staph-Toxin-Ag* based on the *Alere* technology was initially successfully established to study the host immune response to 62 *S. aureus* antigens. Besides a wide repertoire of various known virulence factors of *S. aureus* such as extracellular enzymes, superantigens and complement and coagulation inhibitory proteins, the protein microarray includes more than 20 secreted proteins with so far unknown function. In the present study the protein microarray was used to identify specific interactions of the *S. aureus* proteins to five different human blood plasma proteins known to play a role in the host complement and coagulation system – factor H, fibrinogen, fibronectin, plasminogen and vitronectin. The protein interaction assay was performed using two different sample types: commercially acquired purified host plasma proteins and IgG depleted blood plasma. In the latter case protein-interactions needing additional plasma components for a distinct binding were considered. As positive controls the proteins Efb (**E**xtracellular **F**ibrinogen **B**inding Protein) und Sbi (**S**. aureus **B**inder of **I**gG) were included because of their known binding activity to the complement regulator factor H.
All together we identified 57 *S. aureus* proteins with binding activities to at least one of the five selected human proteins. Among them are 50 proteins interacting with more than one of the plasma proteins. Most interestingly, ten *S. aureus* proteins displayed binding to all five human blood plasma proteins. By comparing the results obtained for the purified human plasma proteins with those for blood plasma sample we got evidence that for at least tree binding activities additional plasma components are necessary. In addition, we learned from this approach that more than ten binding activities might be interfered by plasma components. In follow-up studies using a ligand overlay assay we confirmed specific protein-protein-interactions for 9 out of 10 proteins interacting with plasminogen.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

748/MPP

Analysis of the host response upon infections with *Acinetobacter baumannii* and *Bartonella henselae* using a human *ex-vivo* organ infection model

M. Zander*[1], D. Schmidt[1], W. Ballhorn[1], M. Weidensdorfer[1], A. Reitter[2], V. Kempf[1]
[1]*Goethe University Hospital, Institute for Medical Microbiology and Infection Control, Frankfurt am Main, Germany*
[2]*Hospital Sachsenhausen, Teaching Hospital Goethe-University, Department of Obstetrics and Prenatal Medicine, Frankfurt am Main, Germany*

Animal or cell culture infection models are well established methods to examine the course of infections. However, infection experiments impose an inevitable burden on animals and provide no guarantee that findings can be transferred to humans. Cell culture models, however, are fundamentally limited in their ability to represent the biological context of an infection realistically.
Human *ex vivo* organ infection models (e.g., the human umbilical cord infection model) represent a promising alternative in analyzing pathogen-host interactions. Previous studies demonstrated suitability for elucidating mechanisms of bacterial adherence [mediated via *Bartonella henselae* adhesin A (BadA), or *Acinetobacter baumannii* adhesin (*Ata*)] (Weidensdorfer *et al.*, 2016).
We wanted to investigate whether this organ infection model allows the analysis of the host response upon infections. For this purpose, human umbilical cords were infected with *B. henselae* and *A. baumannii* under dynamic flow conditions. Phorbol 12-myristate 13-acetate (PMA) was used for positive control. The following experimental setting was established: (i) infection/treatment of umbilical cords with bacteria or PMA under blood-flow mimicking conditions, (ii) RNA preparation from umbilical cords and reverse transcription into cDNA and (iii) analysis of the host cell response [via qPCR using the marker genes vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8)]. Results demonstrated a 2.0-fold (1.7 – 2.5) VEGF and a 1.5-fold (1.4 – 1.6) IL-8 gene induction in *B. henselae* infections. *A. baumannii* caused neither VEGF nor IL-8 gene induction (0.4 and 0.6-fold, respectively) whereas *A. baumannii Δata* induced a 2.8-fold IL-8 gene induction (no VEGF-induction; 0.3-fold). PMA induced a VEGF increase of 4.7 (2.4 – 7.0) and an IL-8 increase of 2.7 (1.6 – 3.8).
Our results demonstrate the principle suitability of human *ex vivo* organ infection models in infection research. The use of these models can be expected to return more realistic results and to bring infection research closer to the microenvironmental conditions prevalent within the human body.

References

[1] Weidensdorfer, M., Chae, J.I., Makobe, C., Stahl, J., Averhoff, B., Müller, V., Brandes, R.P., Wilharm, G., Ballhorn, W., Christ, S., Linke, D., Goettig, S., Kempf, V.A.J. Infection of human umbilical cords allows *ex vivo* analysis of endothelial adherence of *Bartonella henselae* and *Acinetobacter baumannii*. Infect Immun. 2016; 28;84(3):711-22.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

749/MPP

Topology mapping of the type three secretion export apparatus proteins

S. Zilkenat*[1], T. Renault[2], M. Erhardt[2], S. Wagner[1]
[1]*Interfaculty Institute of Microbiology and Infection Medicine Tübingen, Cellular and Molecular Microbiology, Tübingen, Germany*
[2]*Helmholtz Centre for Infection Research, Infection Biology of Salmonella, Braunschweig, Germany*

Type three secretion systems are a widespread virulence factor in Gram negative bacteria. The closely related virulence associated (vT3SS) and flagella (fT3SS) systems both contain an inner membrane spanning sub-complex termed the export apparatus. In the vT3SS it translocates, amongst others, effector proteins designated for the host cytoplasm over the inner membrane, in the fT3SS building blocks of the bacterial flagellum. Besides moving proteins over the inner membrane, the export apparatus, made-up of five proteins, is also involved in substrate recognition and in substrate specificity switching. Based on computer predictions, we assessed the topology of three of these transmembrane proteins using substituted cysteine accessibility methods in *Salmonella* Typhimurium SPI-1 vT3SS and flagellum. The position of the transmembrane helices and orientation of the loops of the major export apparatus protein SctV, the switch protein SctU and one of the minor export apparatus proteins SctR were mapped successfully. The prediction could be largely confirmed for SctU and partly for SctV, while the orientation of SctR was reversed. The topology identification of these proteins alongside with recent stoichiometry and interaction studies is an important step in determining the exact placement of the export apparatus in type

III secretion systems and ultimately facilitates elucidation of the function of each component.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

750/MPP

The role of SodM in long persisting *Staphylococcus aureus* clones from cystic fibrosis airways

J. Treffon*[1], D. Block[1], S. Reiss[2], S. Fuchs[2], N. Windmüller[3], L. Langhanki[3], A. Mellmann[3], S. Engelmann[4,5], B. Kahl[1]
[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*
[2]*Ernst-Moritz-Arndt-University, Institute for Microbiology, Greifswald, Germany*
[3]*University Hospital Münster, Institute for Hygiene, Münster, Germany*
[4]*Technical University Braunschweig, Institute for Microbiology, Braunschweig, Germany*
[5]*Helmoltz Center for Infection Research, Microbial Proteomics, Braunschweig, Germany*

Introduction: Cystic fibrosis (CF) patients experience mucus dehydration leading to decreased mucociliary clearance, chronic bacterial infections, lung insufficiency and early death. *Staphylococcus aureus* is one of the earliest pathogens that colonizes and persists in the airways of CF patients.
Objectives: This project aims to detect adaptation mechanisms that facilitate staphylococcal long-term persistence in the CF airways.
Methods: From a longitudinal collection of *S. aureus* strains cultured from respiratory specimens of CF patients clonal short and long persisting *S. aureus* isolates of one patient were chosen for analysis of the cytoplasmic proteome using 2D gel electrophoresis. Five additional strain pairs from other CF patients were used to verify proteomic data by qRT-PCR, functional assays and cell culture experiments.
Results: The proteomic analysis revealed multiple changes in protein abundance. Interestingly, one of the two staphylococcal superoxide dismutases, SodM, was more abundant in the late compared to the first isolate. Functional assays identified higher SodM activity and better growth under oxidative stress conditions for the long persisting clone. In addition, qRT-PCR analysis confirmed the up-regulation of *sodM* in the late isolate. Interestingly, the high expression of *sodM* could also be detected for long persisting clones of four other CF patients. *In vitro* infection assays using A549 airway epithelial cells, the bronchial epithelial CF cell line CFBE41o- and the complemented cell line indicated that clinical isolates with high *sodM* expression seem to be more invasive compared to isolates with lower *sodM* transcription. Interestingly, in internalized bacteria *sodM* is highly expressed while the oxidative stress genes *sodA* and *katA* are not. Currently, we are investigating the role of *sodM* for bacterial intracellular persistence as well as its protective function against phagocytosis by neutrophils. Furthermore, we plan to generate a *sodM* hyperexpression mutant to support our previous findings.
Conclusion: Our results indicate that SodM might be a protein that facilitates the long-term persistence of *S. aureus* in the CF airways by protecting the bacterium against oxidative stress and probably enabling bacterial invasion of host cells.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

751/MPP

The relation of chronic persistent *Staphylococcus aureus* infection and "neutrophil extracellular trap"-formation (NET) in the airways of cystic fibrosis patients

S. Herzog*[1], F. Dach*[1], J. Treffon[1], L. Jahns[1], B. C. Kahl[1]
[1]*Universitätsklinikum Münster, Medizinische Mikrobiologie, Münster, Germany*

Early in cystic fibrosis, *Staphylococcus aureus* is the most common pathogen that colonizes CF lungs with the ability of a long-term persistence. Recurrent infections caused by *S. aureus* are characterized by the dominance of polymorphonuclear neutrophils (PMN). PMNs are capable to form so-called "neutrophil extracellular traps" (NETs) consisting of extracellular DNA and antimicrobial peptides. *S. aureus* can escape NET-driven killing by the secretion of nucleases, which degrade DNA fibers.
This study aims to elucidate mechanisms of *S. aureus* adaption to the hostile environment of CF lungs in context of NET-dependent bacterial killing by PMNs.
To visualize *S. aureus*-induced NET-formation, CF sputum samples were observed via fluorescence microscopy. Isolates from a unique longitudinal *S. aureus* strain collection recovered from the airways of CF patients were used for nuclease testing. Functional analysis of nuclease activity was accomplished by evaluating *S. aureus* isolates on DNA-containing agar plates and by DNA-degradation assays. To quantify the DNA-degrading capacity, a FRET-based fluorescence assay was initiated. Furthermore, transcription of both *S. aureus* nuclease genes (NUC1, NUC2) was analyzed via qRT-PCR. Additionally, the impact of sputum supernatants on *S. aureus* nuclease activity was tested.
In CF sputum samples, NET-related DNA was present in close proximity to *S. aureus*. Testing a multitude of clonal sequential *S. aureus* isolates recovered from a single CF patient, increasing DNA-degradation ability of long-term persisting clones was observed. Also, the extent of DNA-degradation between different phenotypes of *S. aureus* (normal, mucoid and small colony variants) revealed phenotype-depending differences. Such results indicate a time-dependent adaptation of the interaction of persisting *S. aureus* isolates with human PMNs. However, testing strain pairs (early, late) from different CF patients showed an inhomogeneous picture with late isolates being more active, less active or unchanged in activity to early isolates. Interestingly, DNase-activity was inhibited by sputum supernatants of several CF-patients.
Our preliminary results indicate a time-dependent adaption of *S. aureus* to CF airways regarding nuclease activity. Probably due to specific antibodies, host factors are assumed to interfere with the activity of *S. aureus* nucleases. Still, it needs to be clarified if *S. aureus* is or is not able to escape neutrophil attacks by NET-dependent killing and if an escape from NET-dependent killing contributes to long-term persistence of *S. aureus* in CF airways.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

752/MPP

Hypoxia regulates type three secretion in *Salmonella* Typhimurium

S. Walter*[1], R. Gerlach[1], J. Jantsch[2]
[1]*Robert Koch-Institute, P5 - Salmonella Virulence, Wernigerode, Germany*
[2]*Institute for Medical Microbiology and Hygiene, Regensburg, Germany*

Background: Low oxygen tension is a key environmental factor of the gut with declining O2 concentration from proximal to distal. Additionally, a zone of relative oxygenation is found in close spatial proximity to mucosal tissues. Oxygen is a substantial energy source fueling many metabolic pathways and thus has a major impact on bacterial fitness. Therefore, hypoxia not only influences the composition of the microbiota but also the complex

interactions between host and pathogen in multiple and diverse ways. Based on previous findings, we hypothesized that upon *Salmonella* infection an inflamed gastrointestinal tissue displays decreased oxygen tensions which in turn might modulate pathogenicity. The *Salmonella* Pathogenicity Island-1 (SPI-1)-encoded type-three secretion system (T3SS1) is of key importance for efficient invasion into host cells. In this project, we studied the activation of *Salmonella* T3SS1 under distinct oxygen levels, as well as the resulting capability to invade different host cell lines.
Methods: To determine whether hypoxia promotes *Salmonella* invasion into host cells, different epithelial cell lines, such as HeLa, CaCo2 Bbe1, and HuTu80 were infected with *S.* Typhimurium (STM) under different oxygen levels. Besides quantifying intracellular bacteria using gentamicin protection assays, T3SS1 activity was assessed with the help of two SPI-1-dependent fluorescence reporters and subsequent analysis by flow cytometry. To gain further insight into the regulative networks involved in oxygen-dependent modulation of SPI-1 activity a screen of several knock out mutants containing the fluorescence reporters mentioned above was performed. Subsequently, invasion rates were determined to validate these results.
Results: Our experiments revealed an increased SPI-1 dependent invasiveness of wild-type STM under oxygen limited conditions in all tested epithelial cell lines. A T3SS-1 deficient mutant (*ΔinvC*), unable to translocate SPI-1 effectors, served as a negative control. These findings were paralleled by elevated fluorescence signals in hypoxic grown samples assessed by flow cytometry. Interestingly, we identified the two component system ArcAB for being of key importance for the oxygen-dependent modulation of SPI-1 activity and *Salmonella* invasiveness.
Conclusion: In summary, we demonstrate that a hypoxic environment as present in the gut can promote T3SS1-dependent STM virulence. These data imply that under oxygen limitation complex regulatory networks are involved to differentially modulate SPI-1 activity according to distinct oxygen levels. Hence, analysis of *Salmonella*-host interaction under physiologically relevant low oxygen conditions can unravel novel aspects of *Salmonella* pathogenesis.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

753/MPP
The *Legionella pneumophila* protease ProA causes severe damage in human lung tissue explants
S. Thiem*[1], J. Tiefenau[1], J. Jäger[1], S. Marwitz[2,3], C. Kugler[3,4], T. Goldmann[2,3], M. Steinert[1,5]
[1]*TU Braunschweig, Microbiology, Braunschweig, Germany*
[2]*Research Center Borstel, Clinical and Experimental Pathology, Borstel, Germany*
[3]*Airway Research Center North, Giessen, Germany*
[4]*LungenClinic Grosshansdorf, Grosshansdorf, Germany*
[5]*Helmholtz Center for Infection Research, Braunschweig, Germany*

Legionella pneumophila, is the causative agent of Legionnaire´s disease, an acute fibrinopurulent pneumonia. In the environment the bacteria replicate intracellularly within protozoa. Upon human infection *L. pneumophila* mainly replicates intracellularly within alveolar macrophages. The zinc metalloprotease ProA is the main soluble extracellular protein of *L. pneumophila*. This 38 kDa protein is secreted via the type-II secretion system and is homologous to other proteases like elastase of *Pseudomonas aeruginosa* and thermolysin of *Bacillus proteolyticus*. To characterize the contribution of ProA to *L. pneumophila* pathogenicity we infected human lung tissue explants (HLTEs) with the *L. pneumophila* Corby wildtype strain and the *proA*-defective mutant and observed reduced tissue damage and lower replication rates for the mutant. Addition of recombinant purified ProA to HLTEs also caused serious disintegration of the tissue architecture. Interestingly, infections of the macrophage-like cell line THP-1 and the free-living amoeba *Acanthamoeba castellanii* did not reveal effects of ProA on intracellular replication. This demonstrates that ProA is relevant for the extracellular pathogenicity of *L. pneumophila*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

754/MPP
Development of a quick and robust Nanoluc-based type III secretion host cell injection assay
S. Westerhausen*[1], I. Grin[1], S. Wagner[1]
[1]*Institute of Medical Microbiology and Hygiene, Section of Cellular and Molecular Microbiology, Tübingen, Germany*

An increasing number of multi-resistant bacteria has been reported in the last decade, which threatens the effective prevention and treatment of infections. As there are currently only few choices remaining for treatment for multi-drug resistant bacteria, it is crucial to find new alternative approaches. One approach is to discover new substances that are blocking only bacterial virulence factors instead of killing the bacteria. As the bacteria do not require the virulence factor to survive, it is believed that there is less pressure for developing resistance. The Gram negative type III secretion system (T3SS) is such potential target, for which we sought to develop automation-compatible high throughput assays for screening potential bioactive molecules.
For developing such an assay for *Salmonella*, we used the T3SS effector protein SipA and fused it to the recently introduced luciferase Nanoluc. This allowed us to evaluate and quantify the injection of the effector protein into a host cell without extensive culturing, CFU counting or downstream biochemical analysis.
To ascertain the general applicability of this assay, we generated mutant strains deficient in different steps of type III secretion. In addition to the mutants, we also tested a recently discovered compound (C26), which is known to have an effect on the T3SS. By using the developed assay, we could show that compound C26 needs to act on bacteria for a prolonged time period to inhibit type III-dependent effector injection. To address problems with high background luminescence, we are going to test a new split version of the luciferase Nanoluc in the future.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

755/MPP
Panoramic view on *Staphylococcus aureus* biofilm physiology
A. Graf*[1], S. Maaß[1], A. Leonard[2], M. Lalk[2], D. Becher[1], K. Riedel[1]
[1]*University of Greifswald, Institute of Microbiology, Greifswald, Germany*
[2]*University of Greifswald, Institute of Biochemistry, Greifswald, Germany*

Introduction: *Staphylococcus aureus* represents a dangerous opportunistic bacterial pathogen, which is able to cause a wide range of diseases like skin abscesses, bacteraemia and sepsis. In contrast to the planktonic lifestyle associated with these infections, *S. aureus* is also able to form biofilms on host tissues and implants leading to chronic infections found in osteomyelitis, endocarditis and cystic fibrosis patients. Despite the facts that up to 80 % of human bacterial infections are biofilm-associated[1] and Staphylococci are recognized as the most frequent causes of biofilm-associated infections[2], biofilm physiology of *S. aureus* is still largely unexplored.
Objectives: In order to shed light on *S. aureus* biofilm physiology, we aimed to compare protein and metabolic profiles of planktonic and biofilm-associated *S. aureus* cells using *state-of-the-art* multi-omics technologies.
Material and Methods: To this end, we established a flow-through system suited for the cultivation of high amounts of biomass needed for proteomic and metabolic analyses. Biofilm

cultivation using this system allowed us (I) to subsequently analyse the proteome of different subcellular fractions and (II) to monitor concentrations of 39 utilized and secreted metabolites during the time course of cultivation.
Results: Our analyses uncovered significant differences in protein expression and metabolic profiles of planktonic and biofilm-grown *S. aureus* cells. Multiple proteins involved in anaerobiosis, nitrate respiration, arginine deiminase pathway, capsule biosynthesis, osmotic stress response and cardiolipin biosynthesis as well as toxin production have been found strongly induced during biofilm growth. High amounts of fermentation products confirmed our proteome data proving oxygen limitation within a biofilm. Interestingly, well described adhesins, except clumping factor A, are more abundant during planktonic growth – a contrary finding to already published *S. aureus* biofilm concepts.
Conclusion: Here we present the first study combining GeLC-MS and metabolic footprint analysis of *S. aureus* biofilms in a flow through system in order to elucidate *S. aureus* biofilm physiology. Our data contribute to a better understanding of biofilm-associated *S. aureus* infections, an essential prerequisite for the development of novel antimicrobial therapies.

References
[1] Römling & Balsalobre (2012): Biofilm infections, their resilience to therapy and innovative treatment strategies
[2] Otto *et al.* (2008): Staphylococcal biofilms

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

756/MPP
Characterization of isocitrate dehydrogenase Icd/Idh double knockout mutation of *Pseudomonas aeruginos*a with respect to metabolism, virulence and adaptation to CF Lung
M. Arnold[1], N. Crummerl[2], A. Eichner[3], V. Kempf[1], J. Heesemann[2], O. Prazeres da Costa[1], M. Hogardt*[1]
[1]*University Hospital Frankfurt, Medical Microbiology and Infection Control, Frankfurt Main, Germany*
[2]*Ludwig-Maximilians-University Munich, Max von Pettenkofer Institute for Hygiene and Medical Microbiology, Munich, Germany*
[3]*University Hospital Regensburg, Department of Dermatology, Regensburg, Germany*

Pseudomonas aeruginosa can cause a variety of opportunistic human infections including urinary tract infections, wound infections and chronic suppurative pneumonia among cystic fibrosis (CF) patients. During chronic infection of the CF lungs *P. aeruginosa* remarkably adapt to this hostile and heterogeneous environment. In a previous study, we showed that end-stage *P. aeruginosa* CF isolates acquire numerous metabolic adaptations, including upregulation of the isocitrate dehydrogenase (IDH) isoenzymes Icd and Idh. To investigate the potential role of NADPH-dependent Icd (dimeric) and Idh (monomeric) for *P. aeruginosa* virulence, metabolism and habitat adaptation, we compared mutants of Icd (PA14 *icd*::Tn) and Idh (PA14 *idh*::Tn) with a constructed IDH double mutant PA14Δ*icd*Δ*idh*, with respect to their transcriptomic profiles, the expression of several virulence-associated traits and the utilization of nutrients (Biolog). Not surprisingly, due to the interruption of TCA cycle the IDH double mutant showed a significant growth defect both under aerobic and anaerobic conditions. However, growth of the IDH double mutant was supported by supplementation of minimal medium with α-ketoglutarate, glutamate, arginine or ornithine. While the transcriptome of each single mutant showed only minor changes, in the IDH double mutant several transcripts of metabolic enzymes (carbon metabolism), regulators and transport proteins were significantly downregulated. Additionally, IDH double mutant showed defects in motility (swimming, twitching and swarming), elastase- and protease production. In conclusion, our data point to an important link between functioning TCA cycle and virulence of *P. aeruginosa* and indicate that changes in Icd and Idh enzyme activity could play an important role during adaption of *P. aeruginosa* to changing environemental conditions.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

757/MPP
Role of PGRS domain of PE_PGRS5 protein of *Mycobacterium tuberculosis* in ER-stress mediated apoptosis
S. Grover[1], Y. Singh[1], T. Sharma*[1], S. Kohli[1], T. Semmler[2], L. H. Wieler[2], K. Tedin[3], N. Z. Ehtesham[4], S. E. Hasnain[1,5,6]
[1]*Indian Institute of Technology, Kusuma School of Biological Sciences, New Delhi, India*
[2]*Robert Koch Institute, Berlin, Germany*
[3]*Institute of Microbiology and Epizootics, Berlin, Germany*
[4]*National Institute of Pathology, Safdarjang Hospital Campus, New Delhi, India*
[5]*Dr Reddy's Institute of Life Sciences, University of Hyderabad Campus, Hyderabad, India*
[6]*Jamia Hamdard (Hamdard University), New Delhi, India*

The genome of *Mycobacterium tuberculosis* (*M.tb*), the causal organism of Tuberculosis (TB), encodes unique protein family known as the PE/PPE family present exclusively in the genus mycobacterium, with unexplored functions. PE_PGRS5 was found to be highly expressed in lung tissues during a proteomics study. In a high-throughput mutant study, the enrichment of *M.bovis* BCG PE_PGRS mutants (PE_PGRS5, 28, 44, 59) in acidified phagosomes was observed; pointing to the role of PE_PGRS proteins in the arrest of vacuole acidification thus maximizing the intracellular survival. The diverse functional roles of different PE_PGRS proteins in *M.tb* infection thus points towards the importance of highly variable PGRS domain in the survivability of mycobacteria during macrophage infection. We describe the functional significance of PGRS domain of Rv0297, a member of this family. *In-silico* analyses revealed the presence of intrinsically disordered stretches and putative ER localization signals in the PGRS domain of Rv0297. The PGRS domain aids in ER localisation of Rv0297 and causes activation of Unfolded Protein Response as evident from increased expression of GRP78/GRP94 and CHOP/ATF4, leading to disruption of intracellular Ca2+ homeostasis and increased NO and ROS production. The consequent activation of effector caspase-8, resulted in apoptosis of macrophages. These results implicate a hitherto unknown role of the PGRS domain of PE protein family in ER stress-mediated cell death possibly employed by *M.tb* for its dissemination in the later stages of the TB granuloma.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

758/MPP
Molecular and functional analysis of the exotoxin CNF_Y of *Yersinia pseudotuberculosis* YPIII during infection
P. Chaoprasid*[1], J. Schweer[1], W. Heine [1], S. Mühlen[1], P. Dersch[1]
[1]*Helmholtz Center for Infection Research, Molecular Infection Biology, Braunschweig, Germany*

Yersinia pseudotuberculosis is a food-borne pathogen that causes gastroenteritis in humans. The *Y. pseudotuberculosis* strains YPIII and IP2666 of serotype O3 have been shown to produce the cytotoxic necrotizing factor (CNF_Y) toxin. CNF_Y is a single chain AB toxin known to constitutively activate GTPases of the Rho family (Rac-1, Cdc42, RhoA). Small GTP binding proteins of the Rho family has been identified as a common target of bacterial toxins because they play a pivotal role in the regulation of several cellular processes. Purified CNF_Y toxin has been shown to contribute to polymerization of actin leading to formation of stress fiber and impairment of cytokinesis causing multinucleation in HEp-2 cells. In addition, CNF_Y promotes dramatic change cell

morphology by inducing the formation of filopodia, lamellipodia and stress fibres of murine macrophages indicating the activation of Cdc42, Rac1 and RhoA respectively. Moreover, CNF_Y induces the production of reactive oxygen species and pro-inflammatory cytokines, leading to increased inflammation in infected tissues. The inflammation of the examined tissues from mice infected with *Y. pseudotuberculosis* YPIII (WT) was significantly higher in comparison to that induced by the *cnfY* mutant strain. Interestingly, while those mice infected with wild-type YPIII succumbed between days 3 and 6 post infection, mice infected with *cnfY* mutant survived for an extended period after infection. The analysis of type III effector delivery into the host cells during infection was observed by using the reporter dye CCF4/AM. The results demonstrated that CNF_Y enhances the Yop translocation into eukaryotic cells. Therefore, CNF_Y is crucial for virulence of *Y. pseudotuberculosis* during infection.
In further studies, we will focus on the determination and characterization of the functional domains of the CNF_Y protein to better understand its pathogenic mechanism during infection. This finding may help us to better understand the function of the CNF_Y toxin and may prove useful for the therapeutics development. For example, CNF_Y could be used as a drug delivery vehicle.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

759/MPP

Host membrane remodeling upon oxidative stress inhibits *Shigella flexneri* infection

C. Tawk*[1], J. Vogel[1], A. Eulalio[1]
[1]*University of Würzburg, Institute for Molecular Infection Biology, Würzburg, Germany*

Introduction: The interaction of bacterial pathogens with host cells is a complex process whereby the pathogen employs virulence strategies to achieve host colonization, and the host counteracts using defense programs. The encounter of both organisms results in drastic physiological changes leading to stress, which is a prevailing response accompanying infection. Recent evidence suggests that the stress response in the host converges with the innate immune pathways and influences the outcome of infection. However, the contribution of stress and the exact mechanisms of its involvement in the host defense remain to be fully elucidated. In this work, we investigated the role of host stress in infection, using the model bacterial pathogens *Shigella flexneri* and *Salmonella* Typhimurium.
Results and methods: We induced oxidative stress in cultured epithelial cells using arsenite, or stimuli encountered during inflammation such as hydrogen peroxide, hypoxia, and cytokine stimulation. Microscopy analysis and cfu assays showed that *Shigella* binding to host cells is strongly inhibited when oxidative stress is induced prior to infection. We showed that this inhibition is caused by host cell plasma membrane remodeling in response to stress. Microscopy analysis, enzymatic assays, chemical inhibitors, and knockdown using siRNAs revealed that the stress-dependent activation of the acid sphingomyelinase (ASM) and the neutral sphingomyelinase (NSM) leads to the depletion of sphingolipid-rafts in the membrane. Moreover, intracellular *Shigella* replication also causes the activation of sphingomyelinases across adjacent cells, inhibiting the invasion by extracellular bacteria. Interestingly, *Salmonella* adhesion was not affected by stress induction, since it uses flagellar motility to successfully gather at the remaining membrane rafts.
Conclusion: Overall, our findings demonstrate that epithelial cell membrane remodeling in response to oxidative stress contributes to the defense against non-motile bacteria. Importantly, upon *Shigella* infection this response can be elicited also in bystander cells, preventing further bacterial infection. However, bacterial pathogens with flagellar motility, such as *Salmonella*, are able to circumvent this defense mechanism.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

760/MPP

Comparative proteomic analyses of Sigma family across mycobacteria assert their role in virulence and pathogenicity

K. Sharma*[1], P. Gupta[1], V. raveendran[2], J. A. Sheikh[3], N. Ehtesham[3], S. Hasnain[1,4,5]
[1]*IIT Delhi, Kusuma School of Biological Sciences, New Delhi, India*
[2]*Indian Institute of Science Education and Research, Kolkata, India*
[3]*National Institute of Pathology, Safdarjang Hospital Campus, New Delhi, India*
[4]*Dr Reddy's Institute of Life Sciences, Hyderabad, India*
[5]*Jamia Hamdard University, New Delhi, India*

Tuberculosis (TB) is a leading human infectious disease caused by *Mycobacterium tuberculosis*. Bacteria counter the immune response generated by infected host by transcriptional switching and metabolic remodelling. Therefore, a deeper insight into the pathogenicity and infection biology of mycobacteria can be likely achieved by studying how mycobacteria are able to remodel or regulate its gene expression. One of the important regulatory gene family is the sigma family which helps in transcription initiation process and in expression of specific regulons in response to specific stimuli. Our comparative proteomic analysis of sigma family showed variation in number of sigma factors and their target genes across different mycobacterial species highlighting the changes acquired in functional domains of sigma family as we move from non-pathogens to pathogens thereby partly also explaining the evolution of pathogenicity across mycobacteria. A multiple sequence alignment across all sigma factors of *M.tb* H37Rv showed the presence of a few conserved motifs. Transcriptional mapping of regulatory interactions of sigma family and their targets in *M.tb* H37Rv identified certain common denominators which can be used as putative drug targets against TB.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

761/MPP

Influence of SigB on regulation of virulence factors of *Staphylococcus saprophyticus*

J. Busse*[1], M. Korte-Berwanger[1], S. G. Gatermann[1]
[1]*Ruhr-Universität Bochum, Department of Medical Microbiology, Bochum, Germany*

Introduction: *Staphylococcus saprophyticus* is an opportunistic pathogen and a common cause of urinary tract infections. Several virulence factors have been described, e.g., a urease, the surface proteins Aas, SdrI and UafA, a surface-associated lipase Ssp and a D-serine-deaminase DsdA. Since D-serine is prevalent in urine and has a toxic or bacteriostatic effect on many bacteria, possession of the D-serine-deaminase seems to be an important adaption for colonization of the urinary tract. It was shown that the lipase Ssp is upregulated in presence of D-serine. However, we do not know anything about the mechanisms of regulation of the D-serine-deaminase or any other virulence factor. Bacteria often use regulation systems to modulate expression of virulence factors as a reaction to certain environmental influences. From the data on Ssp it may be announced that D-serine has an influence on the expression of a regulation system. For *Staphylococcus aureus* it is known that the alternative sigma-factor SigB plays a major role in regulation of virulence factors. Genome analysis showed that *sigB* also exists in *S. saprophyticus*. The physiological significance of SigB and the influence of D-serine on this system should be investigated by construction of a *sigB* knock-out

mutant, followed by comparative physiological tests and real time qRT-PCR.
Methods: Cloning of a *sigB* knock-out construct was accomplished by use of a yeast recombination system. To this end, the erythromycin cassette and a 500 bp fragment upstream and downstream of *sigB* was amplified and transformed with the linearised shuttle vector pRS426 into yeast strain PJ69-4a. The assembled mutagenesis construct was cloned into the temperature sensitive replacement shuttle vector pBT2. Plasmids were purified from *E. coli* DH5α and transformed into *S. saprophyticus* strain 7108 by protoplast transformation, followed by a plasmid curing step.
Results and Conclusion: A *sigB* knock-out mutant was constructed. Expression of virulence factors was investigated by different physiological assays such as urease activity testing, D-serine-deaminase activity testing, lipase activity testing or binding and biofilm assays. The *sigB* knock-out mutant showed lower urease activity and lower D-serine deaminase activity, when grown under the influence of 20 mM D-serine. This indicates that the SigB system is involved in virulence factor regulation and influenced by the presence of D-serine as an environmental signal.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

762/MPP
A chloride dependent mannitol-1-phosphate dehydrogenase/phosphatase in the opportunistic pathogen *Acinetobacter baumannii*
P. König*[1], S. Zeidler[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt / Main, Germany*

Introduction: *Acinetobacter baumannii* is an emerging pathogen characterized by its ability to withstand adverse life circumstances. Outstanding, among others, is its extreme desiccation resistance. We hypothesize that compatible solutes play a role in desiccation resistance in *A. baumannii*. Mannitol is a very unusual compatible solute found in its relative, *Acinetobacter baylyi*, where it is synthesized from fructose by a novel bifunctional mannitol-1-phosphate dehydrogenase/phosphatase (MtlD).
Objectives: The goal of this study was to determine whether mannitol is synthesized by *A. baumannii* as well and how the synthesis is regulated.
Materials & Methods: Mannitol was determined by NMR. A markerless deletion mutant of *mtlD* in *A. baumannii* ATCC 19606 was created with single homologous recombination and segregation of the plasmid via *sacB*. The *mtlD* gene of *A. baumannii* was expressed in *E. coli* and the enzyme was purified by affinity chromatography and characterized biochemically.
Results: *A. baumannii* produced glutamate and mannitol at high salt. No mannitol could be detected in the *mtlD* deletion mutant, indicating that MtlD is a mannitol dehydrogenase. As expected, MtlD reduced NADPH in the presence of the substrate fructose-6-phosphate. The enzymatic activity was clearly salt dependent. High activities could be measured with NaCl, whereas other sodium salts did not stimulate. This led us to analyze the role of chloride in MtlD activity. Indeed, with keeping the ionic strength constant, increasing Cl^--concentrations led to increasing activities with a maximum at 500 mM.
Conclusion: *A. baumannii* synthesizes mannitol as compatible solute. The biosynthetic key enzyme is strictly chloride-dependent. Cl^--dependent processes in prokaryotes are very scarce and a potential role of chloride in pathogenesis of *A. baumannii* is discussed.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

763/MPP
A novel role of a major cell wall glycopolymer in *Staphylococcus aureus* virulence
S. Wanner[1], J. Schade*[1], D. Keinhörster[1], S. E. George[1], C. Wolz[1], J. Kolata[2], D. Kretschmer[1], B. Bröker[3], C. Weidenmaier[1]
[1]*Institut für Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*
[2]*Medical Microbiology UMC, Utrecht, Netherlands*
[3]*Universitätsmedizin Greifswald , Institut für Immunologie und Transfusionsmedizin , Greifswald, Germany*

Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) have been under intensive investigation during the last decade due to their fast epidemic spread and their enormous virulence potential which exceeds that of the traditional hospital-acquired strains (HA-MRSA). Skin and soft tissue infections (SSTIs) account for ninety percent of CA-MRSA infections, ranging from mild skin infections to severe abscesses. We report here a novel mechanism that contributes to the elevated virulence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). CA-MRSA are causing a severe pandemic of mainly skin and soft tissue infections and some of the underlying pathomechansisms and virulence factors have been studied in detail. So far, increased expression of core-genome-encoded virulence determinants, such as phenol-soluble modulins, α-toxin and acquisition of phage-encoded genes have been correlated with virulence of CA-MRSA strains. However, the relative role of virulence factors in CA-MRSA virulence is still debated and several lines of evidence hint to the involvement of additional so far unknown factors. When we analyzed cell wall composition of different *S. aureus* strain backgrounds we discovered that especially highly virulent CA-MRSA exhibited severely increased amounts of cell wall attached teichoic acids (WTA). We have shown before that this major glycopolymer of the staphylococcal cell wall can activate CD4-T cells in an MHC II dependent manner and plays an important role in the development of staphylococcal skin abscesses. Since CA-MRSA are the predominant isolates in staphylococcal skin infections, we compared the capacity of cell wall fractions isolated from highly pathogenic CA-MRSA and less virulent strains to induce abscess formation in a subcutaneous mouse model. Abscess induction indeed correlated strongly with the amount of cell wall incorporated WTA. We found that CA-MRSA specifically up-regulated WTA content (WTAhigh) and thus were more active in inducing abscess formation via a T cell dependent mechanism than cell walls from *S. aureus* strains with a WTAlow phenotype. We could demonstrate that the WTAhigh phenotype depends on an increased expression of an integral WTA transporter gene and thus on an increased capacity of WTAhigh strains to transport the nascent WTA chain to the outside of the bacterial cell prior to incorporation into the cell wall. Interestingly, the up-regulation of WTA transporter expression was a function of increased Agr-activity. Agr is a major virulence regulator and high Agr-activity was a hallmark of the WTAhigh strains we analyzed when compared to WTAlow strains. Taken together, we present here a novel concept of staphylococcal virulence that is based on the increased biosynthetic activity of a major staphylococcal cell wall polymer.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

764/MPP

Role of betaine/choline/carnitine transporters (BCCTs) in osmostress protection of *Acinetobacter baumannii*

J. Breisch*[1], I. Waclawska-Krzeminski[1,2], B. Averhoff[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt / Main, Germany*
[2]*University Regensburg, Biophysics & Biophysical Chemistry, Regensburg, Germany*

Introduction: In the past decades *Acinetobacter baumannii* emerged as a nosocomial pathogen and obtained high clinical relevance due to its increasing multidrug resistance. *A. baumannii* is very well adapted to host cells and can growth under iron- and phosphate-limitation and survive low pH-value and oxidative-stress. Furthermore, *A. baumannii* colonizes almost any surface and persists dessication and moisture which indicates powerful osmostress response mechanisms. To counteract high salt concentrations, many bacteria take up compatible solutes by betaine/choline/carnitine transporters (BCCT). However, information with respect to the role of compatible solutes in osmostress protection of *A. baumannii* is scarce.
Objectives: The aim was to identify and characterize BCCTs involved in osmostress protection of *A. baumannii*.
Materials & Methods: A markerless mutagenesis system was applied to generate BCCT mutants. The role of the BCCTs in osmoprotection was analysed by mutant studies. Furthermore, the BCC transporters were heterologously expressed, purified and biochemically characterized.
Results: Inspection of the genome of *A. baumannii* ATCC 19606 revealed four potential transporters, BetT1, BetT2, BetT3 and BetTX belonging to the BCCT family. Preceding functional studies of BCC transporters of the non-pathogenic *A. baylyi* and sequence analyses suggest that BetT1 and BetT2 act as choline specific transporters whereas BetT3 is suggested to mediate betaine transport. Mutant studies revealed that BetTX mediates choline transport under high-salt conditions in the presence of choline.
Conclusions: The BCC transporters are involved in the persistence of *A. baumannii* under hyperosmotic conditions. The presence of multiple choline specific transporters suggests that different BCCTs have distinct roles in adaption of *A. baumannii* to osmostress and to host tissues comprising a large amount of phosphatidylcholin.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

765/MPP

Antibactericidal activity of C6-ceramide and C6-ceramide analogs against the Gram-negative bacterium *N. meningitidis*

J. Becam*[1], A. Burgert[2], T. Walter[3], M. Sauer[2], J. Seibel[3], A. Schubert-Unkmeir[1]
[1]*Institute of Hygiene and Microbiology, Wuerzburg, Germany*
[2]*University Wuerzburg, Department of Biotechnology & Biophysics, Würzburg, Germany*
[3]*University Wuerzburg, Institut fuer Organische Chemie, Würzburg, Germany*

Question: *Neisseria meningitidis*, an obligate human pathogen is often found in the nasopharynx as an asymptomatic colonizer. Within its ecological niche meningococci have to encounter different host immune effectors including antimicrobial activity of sphingolipids and fatty acids. The latter are involved in the physical barrier as well as in immunologic barrier function.
Certain fatty acids and sphingoid bases found at mucosal surfaces are known to have antibactericidal activity and are thought to play a more direct role in innate immunity against bacterial infection. Interestingly, *N. meningitidis* exhibits an intrinsic fatty resistance. Herein, we analysed the antibacterial activity of sphingolipids, including respectively short and long-chain ceramides (C6-cer; C16-cer).
Methods: In order to test the effect of ceramides on meningococci commercially available, C6-ceramide and C16-ceramide were employed. Antimicrobial activity against meningococci was determined by growth kinetics. In addition, broth microdilution assays were used to determine the minimal inhibitory concentration (MIC). To visualize uptake and transport of ceramides within bacteria, azido-functionalized ceramides (α-azido-C6-cer, ω-azido-C6-cer, α-azido-C16-cer and ω-azido-C16-cer) were synthesized. Acquisition of the fluorescent click-labeled azido-functionalized ceramides was determined by flow cytometry and visualized using *direct* Stochastic Optical Reconstruction Microscopy (*d*STORM). In addition, toxicity on host cells was evaluated by annexin V and propidium iodide staining. Biological function of ceramide uptake was assessed determining adherence and invasiveness to host cells by gentamicin protection assays.
Results: Short-chain ceramide as well as C6-ceramide analogs were antibactericidal against *N. meningitidis*, whereas long-chain C16 ceramides were non-toxic. Kinetic assay showed that killing of *N. meningitidis* occurred within 1 h. Ω-azido-C6-cer was more toxic than α-azido-C6-cer. Interestingly, at a bactericidal concentration, short-chain C6-ceramide had no significant toxic effect on host cells. However, uptake of both C6-ceramide as well as C16-ceramide in a concentration below the MIC could increase invasiveness. Bacteria fed with ω-azido-C16-ceramide and clicked with specific dyes suggested, by double staining, this molecule is incorporated within the outer membrane and might be accumulated by bacteria as intracellular inclusion.
Conclusions: Our results indicate a bactericidal effect of short-chain C6 ceramides on *N. meningitidis* at a non-toxic concentration for mammalian cells. In addition, we demonstrated that bioorthogonal functionalized ceramide probes and super-resolution can be used to visualize their incorporation.
Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

766/MPP

Functionality of a holin-endolysin cassette within a pathogenicity island of *Yersinia enterocolitica*

K. Springer*[1], T. M. Fuchs[1,2]
[1] *Chair for Microbial Ecology, TUM, Department of Biosciences, Wissenschaftszentrum Weihenstephan, Freising, Germany*
[2]*Friedrich-Loeffler-Institut, Jena, Germany*

The *Yersinia enterocolitica* strain W22703 exhibits insecticidal and nematocidal activity. This toxicity is generated by proteins of the toxin complex (Tc) being encoded by the pathogenicity island Tc-PAIYe. The island genes code for two regulators, TcaR1 and TcaR2, and for the type A, type B and type C toxin subunits and are expressed at environmental temperature, but silenced at 37°C. The LysR-like regulator TcaR2 is essential and sufficient to activate toxin gene transcription at low temperatures. TcaR2 is autoregulated, it is unstable at body temperature and its expression is repressed by TcaR1 (Starke et al., 2013). The *Yersinia* modulator A (YmoA) is an antagonist of TcaR2 and represses the toxin gene transcription at 37°C (Starke & Fuchs, 2014). The three subunits form a tripartite ABC-type toxin complex, with subunit C being translocated into the host cell causing cell death by its ADP-ribosyltransferase activity.
In addition to the toxin complex proteins the gene island contains four open reading frames related to phage genes. These genes are highly conserved in all Tc-PAIs known so far, and two of them show strong homologies to holins and endolysins. Within this study we investigated the biological functionality of these phage genes.
To test the biological function of this holin/endoylsin cassette, growth experiments were performed at 37°C and 15°C with

various *Y. enterocolitica* strains and *E. coli* carrying the two genes on an arabinose-inducible vector. Using zymography we analyzed the ability of the yersinial endolysin to cleave the bacterial cell wall. We also wanted to explore if the putative holin indeed exhibits a pore-forming activity.
The overproduction of holin and endolysin resulted in cell lysis of *E. coli* both at 37°C and 15°C, demonstrating a temperature-independent biological activity of the lysis cassette. For a variety of *Y. enterocolitica* biovar strains, a broad spectrum of efficacy was observed, ranging from full cell lysis (biovar 2) to no impairment (biovar 3). Interestingly, the holin/endolysin cassette is also active against its own host cell at 37°C, but not at 15°C. Recent data indicate that this temperature-dependent activity is due to their proteolytic degradation by protease Lon at 15°C. We confirmed the functionality of the yersinial endolysin via zymograms and observed high substrate specificity. The strongest activity was observed against W22703 cells (100%), and a weaker activity against *Y. ruckeri* (32%) and *E. coli* cells (5%). We demonstrated the holin-like activity of the putative *Yersinia* holin by successfully complementing a λ phage within *E. coli* that carries a mutated holin gene. The expression of the yersinial holin induced complete bacterial lysis within less time compared to the λ phage holin.
These data demonstrate that the holin and endolysin genes encode for functional proteins which are able to lyse their own host cell under appropriate conditions.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

767/MPP
Structural characterization of a multi-functional virulence factor of *Corynebacterium diphtheriae*
C. Azevedo Antunes*[1], D. Weerasekera[1], I. Henderson[2], A. L. Mattos Guaraldi[3], A. Burkovski[1]
[1]*Friedrich-Alexander Universität Erlangen-Nürnberg, Professur für Mikrobiologie, Erlangen, Germany*
[2]*University of Birmingham, Institute of Microbiology and Infection - IMI, Birmingham, United Kingdom*
[3]*Rio de Janeiro State University, Laboratory of Diphtheria and Corynebacteria of Clinical Relevance-LDCIC, Faculty of Medical Sciences, Rio de Janeiro, Brazil*

Corynebacterium diphtheriae is a microorganism of global significance for being the etiologic agent of diphtheria and systemic infections. The diphtheria bacillus has been classically described as an extracellular pathogen. However, a number of studies revealed its ability to invade epithelial cells, indicating a more complex pathogen-host interaction. The molecular mechanisms controlling and facilitating internalization of *C. diphtheriae* are poorly understood. Comparative proteomic analysis *in silico* and *in vitro* suggested the importance of the protein DIP0733 and indicated its ability to interact with matrix proteins and cell surfaces, participating in cellular internalization and induction of cell death. Besides seven transmembrane helices, we identified short linear motifs and structural elements of DIP0733 with putative importance in virulence using bioinformatic approaches. Interestingly, a C-terminal coil-coiled region of the protein was particularly considered important, since it was found only in DIP0733 homologs of pathogenic but not in non-pathogenic corynebacteria. For the bioinformatically analyzed structural elements, a cloning strategy was used as further step to test these elements for their biological function. Expression plasmids with different truncated DIP0733 variants were tested in host-pathogen interaction assays. Epithelial cells and macrophage infections revealed a reduced effect of truncated forms of DIP0733, suggesting an important role of the coil-coiled region to interact with the host cell. Moreover, to further analyze the structure of DIP0733, its sequence was cloned into an *E. coli* expression system. Different protocols for DIP0733 expression has been optimized and the purified protein was obtained in the membrane fraction for antibody production and preparation for crystallography.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

768/MPP
Generation and functional characterization of a *Salmonella* Typhimurium mutant lacking seven methyl-accepting chemotaxis genes
S. Hoffmann[1], C. Schmidt[1], S. Walter[1], J. K. Bender[2], R. G. Gerlach*[1]
[1]*Robert Koch Institute, Project Group 5, Wernigerode, Germany*
[2]*Robert Koch Institute, Division 13, Wernigerode, Germany*

Background: Site-directed scarless mutagenesis is an essential tool of modern pathogenesis research. We previously described a two-step protocol in *Salmonella enterica* serovar Typhimurium (STM) based on the λ Red recombinase-catalyzed integration of a selectable antibiotics resistance marker and subsequent replacement of this cassette[1]. Selection of markerless mutants is achieved by expressing the meganuclease I-SceI which induces double strand breaks in bacteria harbouring the resistance cassette. We sought to improve this method in a way to enable multiple sequential mutagenesis steps in a single strain with a simpler and faster protocol.
Methods: The new plasmid pWRG730 enables heat-inducible expression of the λ Red recombinase and tet-induced production of the I-SceI meganuclease. Together with a newly designed kanamycin resistance cassette, pWRG730 was used to sequentially delete 7 methyl-accepting chemotaxis (MCP) genes in STM strain NCTC 12023. We used PCR to verify the correctness of the mutants at each step of the deletion process. The final mutant lacking all 7 MCP genes was subjected to whole genome sequencing (WGS). For functional characterization of the 7 MCP mutant swarming assays on semi-solid agar and a HeLa cell infection model was applied.
Results: The improved system enabled the fast and efficient generation of a collection of MCP mutant bacteria finally resulting in a 7 MCP scarless knockout strain. Each step of this process was successfully confirmed by PCR. The correct gene deletions in the 7 MCP mutant could be verified by WGS. Prolonged expression of the λ Red recombinase system bears the risk to be mutagenic. Here, WGS showed that tight restriction of λ Red expression as implemented in our protocol prevented unwanted mutations. Swarming assays revealed for the 7 MCP mutant, as expected, a similar phenotype as a non-chemotactic *cheY* mutant. Chemotaxis of this mutant was partially restored by plasmid-driven expression of the single MCP CheM/Tar. To further characterize the impact of chemotaxis on the interaction of STM with host cells all MCP mutants were tested in a HeLa cell infection model. Here, the *cheY* mutant and all mutants lacking *cheM* showed increased invasion rates. The phenotype of the 7 MCP mutant was reversed by complementation with an expression plasmid harbouring *cheM*.
Conclusion: Our optimized scarless deletion protocol enables efficient and precise manipulation of the *Salmonella* genome. As demonstrated with WGS, multiple subsequent mutagenesis steps can be done without introducing unwanted mutations. The sequential deletion of 7 MCP genes revealed a significant role of CheM for the interaction of STM with host cells which might give new insights into mechanisms of *Salmonella* host cell sensing.

References
[1] Blank K. *et al*. PLOS One (2011)

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

769/MPP

Regulatory mechanisms of staphylococcal persistence

A. Siegmund*[1,2], F. Gratani[3], C. Wolz[3], S. Niemann[1], B. Löffler[2,4], L. Tuchscherr [2,4]
[1]*Universitätsklinikum Münster, Institut für Medizinische Mikrobiologie, Münster, Germany*
[2]*Universitätsklinikum Jena, Institut für Medizinische Mikrobiologie, Jena, Germany*
[3]*Universitätsklinikum Tübingen, Institut für Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*
[4]*Universitätsklinikum Jena, Center for Sepsis Control and Care, Jena, Germany*

Introduction: *Staphylococcus aureus* is a frequent pathogen that causes chronic and therapy-refractory infections such as osteomyelitis. *S. aureus* can act as a facultative intracellular pathogen capable of invading multiple types of host cells and persist in the intracellular environment for long-time periods that is associated with phenotype switching to small colony variants (SCVs). Yet, the signals, stress factors and mechanisms that induce persistence and phenotype switching remain unknown.
Aim: This project was designed to investigate the impact of the stringent stress response on *S. aureus* SCV-formation and persistence. The stringent stress response is initiated by a rapid synthesis of the alarmones ppGpp and pppGpp, mediated by RSH, RelP and RelQ enzymes.
Methods and Results: Single, double and triple staphylococcal mutants were generated in *S. aureus* strains LS1 and USA300 to decrease levels of enzymes involved in the stringent response (Δ*rsh*; Δ*rsh/relP*, Δ*rsh/relQ*, Δ*rsh/relP/relQ*). First experiments were performed to study the bacterial uptake by endothelial cells and cell death. No differences were observed between wild type (WT) and stringency response mutants in both backgrounds. The mutants were tested also for persistence and formation of SCVs in EA.hy926 (endothelial cells) culture systems. Seven days after infection of endothelial cells the number of intracellular CFU/ml recovered did not differ between WT and mutant strains. Moreover, the percentage of SCVs formed was not statistically significant in any of the tested strains. To analyse possible escape from intracellular degradation mechanisms phagosome escape in A549 cells (epithelial cells) was tested. The percentage of bacteria in cytoplasm did not differ in any of the tested strains.
Conclusion: In conclusion, we did not find an impact of the stringent response on acute phase of infection as cell death and cell invasion were not affected. Moreover, it was not found a relation between long-term intracellular persistence of *S. aureus* in endothelial cells and stringency response, although the system was reported to contribute to intracellular persistence in other pathogens.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

770/MPP

***Staphylococcus aureus* pathogenesis: from sepsis to hematogenous chronic bone infections.**

C. Pöllath*[1], B. Löffler[1], L. Tuchscherr de Hauschopp[1]
[1]*University Hospital of Jena , Institute of Medical Microbiology, Jena, Germany*

Introduction: *Staphylococcus aureus* is a gram positive bacterium, which is able to colonize epithelial surfaces of healthy individuals, as well as causing infections, ranging from local to life threatening systemic infections, like sepsis. Moreover *S. aureus* can escape from the blood stream and establish infection foci in surrounding tissue, such as bones. Once the bacteria invaded the bone cells, they are able to cause an acute infection by expressing virulence factors or to remain silent and persist inside the cells. Especially this chronic osteomyelitis is exceedingly difficult to treat and often requires drastic surgical procedures.
Aim/Question: The aim of this work was to detect characteristic features of isolates that cause hematogenous osteomyelitis.
Methods / Results: To elucidate the connection of sepsis and chronic bone infections, a strain collection was established. The isolates were assigned to four different clinical categories: nasal colonization of healthy persons, sepsis without metastasis, osteomyelitis with and without hematogenous origin. Those groups were genotyped for their clonal complex, resistance profile and different virulence and adhesin genes. Only minor differences between the groups could be observed whereas the variation within the single groups was high.
The infection with *S. aureus* is characterized by initial adhesion to host cells followed by invasion and either killing of the cell or persistence in that. These different stages of infection were mimicked with various *in vitro* experiments. The ability to produce biofilm is coupled with the expression of adhesins. To asses this trait, the strains were tested for their biofilm formation on plastic surface after 24h, though the differences between the groups were not significant. To test the invasiveness and cytotoxicity, flow cytometric assays were used, which resulted in no significant differences regarding the ability to invade host cells. In contrast, the group of non-hematogenous osteomyelitis showed a significant lower cytotoxicity, compared with the isolates belonging to the colonizing and sepsis group. To picture the cytotoxicity of the strains in more detail, a hemolysis assay was performed. A significant difference was found between the group of nasal colonizing strains and the hematogenous osteomyelitis group, whereas the latter showed a higher hemolysis grade of sheep blood erythrocytes.
Conclusion: The preliminary data suggest that strains, which are related to the bloodstream, tend to show a higher cytotoxicity regarding their ability to destroy osteocytes and erythrocytes. A more detailed understanding of how *S. aureus* interacts with the host as part of the disease process could ultimately lead to potential targets for new therapeutics to help control staphylococcal infections.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

771/MPP

In vivo analysis of the assembly of the complete type III secretion needle complex.

N. Singh*[1], T. Dietsche[1], S. Wagner[1]
[1]*Institut für Med. Mikrobiologie und Hygiene, Section of Cellular and Molecular Microbiology, Tübingen, Germany*

Many pathogenic Gram-negative bacteria use type III secretion systems (T3SS) to secrete effector proteins into target host cells. T3SS are composed of over 20 different proteins and build a membrane spanning multi-megadalton complex of about 200 subunits. Based on previous work, a bipolar nucleation model of needle complex assembly was proposed, which indicates an inherent quality control mechanism for the correct formation of T3SS: nucleation at the inner membrane ensures that only export apparatus containing MS rings are formed, hence the system is secretion competent. Nucleation in the outer membrane by the secretin ring assures penetration at the outer membrane by the growing needle. Since secretion and penetration competence is crucial for the functionality of the T3SS and thereby virulence and infection, the precise orchestration of assembly processes is vital for the survival of pathogens harboring T3SSs. In order to analyze the assembly pathway of the complete T3SS and test the hypothesis of bipolar assembly, we need to address the following questions: how does the export apparatus assemble and gets integrated in the MS-ring? How do inner and outer membrane sub-complexes integrate? How do the cytoplasmic components assemble onto the remainder of needle complex and how does their assembly reflect the function of the secretion system?

We use a site-specific *in vivo* photo-crosslinking system based on the artificial amino-acid para-benzoyl phenylalanine (pBpa) in order to identify signature crosslinks characterizing all occurring protein-protein interactions of the secretion competent machine and deduce its assembly pathway based on the dependence of the observed crosslink on presence of individual NC components. Here we present the proof of concept and the current state of the project.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

772/MPP

Shiga-like toxin as a novel virulence factor in *Corynebacterium diphtheriae* and *Corynebacterium ulcerans*

D. Weerasekera*[1], C. Azevedo Antunes[2,1], A. L. Mattos Guaraldi[2], A. Burkovski[1]
[1]*Friedrich-Alexander Universität Erlangen-Nürnberg, Professur für Mikrobiologie, Erlangen, Germany*
[2]*Rio de Janeiro State University, Laboratory of Diphtheria and Corynebacteria of Clinical Relevance-LDCIC, Faculty of Medical Sciences, Rio de Janeiro, Brazil*

Shiga toxins are a group of type 2 ribosome-inactivating proteins produced in *Shigella dysenteriae* and related bacteria. Interestingly, a similar protein has been found in *Corynebacterium ulcerans*. The ribosome binding protein (Rbp) of *C. ulcerans* 809 represents a putative virulence factor, putatively inhibiting the protein biosynthesis in host cells. In this study, we have identified several conserved amino acid and catalytic residues in the Rbp of *Corynebacterium diphtheriae* strain HC04 with striking structural similarity to the Rbp in *C. ulcerans* 809. The aim of this project was to characterize the expression of Shiga-like toxin in *C. diphtheriae* and *C. ulcerans* strains and its role of interaction with host cells. An insertion mutant of strain *C. diphtheriae* HC04 was generated using standard techniques. First, a *Caenorhabditis elegans* model system was used to investigate the pathogenicity and virulence factors associated with *C. diphtheriae* and *C. ulcerans* strains. In a nematode survival assay, it was observed that in contrast to the wild type strains, the overexpression strains of *C. diphtheriae* HC04 and *C. ulcerans* 809 were more detrimental to *C. elegans*. Furthermore, the transepithelial resistance (TER) of Detroit562 cell monolayers was measured to find out if *C. ulcerans and C. diphtheriae* are able to damage host cells. In this study, we used *Salmonella* Typhimurium NCTC 12023 as a positive control which caused a fast breakdown of TER while the negative control without bacteria showed an even, increasing TER which later remained constant. Compared to *S.* Typhimurium, effects of monolayers caused by the strains *C. ulcerans* 809, *C. diphtheriae* HC04 and their overexpression strains were considerably slower. Future work relies on quantitative analysis of adhesion and internalization behavior of the *C. diphtheriae* strains on cells of the human epithelial cell line HeLa and the survival of the strains after internalization by human macrophages THP-1.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

773/MPP

Excreted cytoplasmic proteins contribute to pathogenicity in *Staphylococcus aureus*

P. Ebner*[1], J. Rinker[1], A. Luqman[1], P. Popella[1], S. Reichert[1], F. Götz[1]
[1]*Univerität Tübingen, Microbial Genetics, Tübingen, Germany*

Excretion of cytoplasmic proteins in pro- and eukaryotes, also referred to as 'non-classical protein export', is a well-known phenomenon. However, comparatively little is known about the role of the excreted proteins relating to pathogenicity. Here the impact of two excreted glycolytic enzymes, aldolase (FbaA) and glyceraldehyde 3-phosphat dehydrogenase (GapDH) on pathogenicity was investigated in *Staphylococcus aureus*. Both enzymes bound to certain host matrix proteins and enhanced adherence of the bacterial cells to host cells but caused a decrease of host cell invasion. FbaA and GapDH also bound to the cell surface of staphylococcal cells by interaction with the major autolysin Atl that is involved in host cell internalization. Surprisingly, FbaA showed a high cytotoxicity to both MM6 and HaCaT cells, while GapDH was only cytotoxic for MM6 cells. Finally, the contribution of external FbaA and GapDH to *S. aureus* pathogenicity was confirmed in an insect infection model.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

774/MPP

Identification of novel phospholipases A in *Pseudomonas aeruginosa*

A. J. Weiler*[1], C. H. Strunk[1], F. Kovacic[1], K. E. Jaeger[1,2]
[1]*Heinrich-Heine Universität Düsseldorf, Institute of Molecular Enzymtechnology, Jülich, Germany*
[2]*Forschungszentrum Jülich GmbH, 2. Institute of Bio- and Geosciences IBG-1: Biotechnology, Jülich, Germany*

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen infecting immunocompromised humans (cystic fibrosis, AIDS and cancer patients) with high mortality rates1. This bacterium is known to produce an array of different lipolytic and proteolytic enzymes and different types of adhesins, which mediate pathogenicity of *P. aeruginosa*2. Although, many virulence associated genes have been identified, the function of 40% of all *P. aeruginosa* genes is still unknown.
Here, we aimed to identify, purify and characterize additional and so far unknown lipolytic enzymes possibly involved in pathogenicity of *P. aeruginosa*. By now, two novel and yet unknown membrane bound esterases were identified *via* zymographic analysis. Furthermore, we study two putative GDSL-lipases of *P. aeruginosa*. So far, we have identified PlaB, a novel phospholipase A of *P. aeruginosa*, which upon expression in *E. coli* resulted in functional enzyme with phospholipase A activity. Experiments with purified PlaB and with a *plaB* gene deletion strain of *P. aeruginosa* should reveal its role for virulence of *P. aeruginosa*.

References

[1] Van Delden, C. and B. H. Iglewski (1998) Emerg Infect Dis 4: 551-560.
[2] Jaeger, K.-E. and F. Kovacic (2014) Methods Mol Biol 1149: 111-134.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

775/MPP

Proteomic analyses reveal an extensive impact of *Salmonella*-induced filaments on the nutritional supply inside the SCV

J. Noster*[1], T. C. Chao[2], T. Reuter[1], M. Hensel[1], N. Hansmeier[1]
[1]*University of Osnabrueck, Microbiology, Osnabrück, Germany*
[2]*University of Regina, Institute of Environmental Change & Society, Regina, Canada*

Background: *Salmonella enterica* serovar Typhimurium (STM) has the unique ability to transform the host cell endosomal system into a tubular network known as *Salmonella*-induced filaments (SIF). Translocation of SPI2-T3SS effector proteins mediates the formation of this network. Recent work demonstrated that SIF formation is prerequisite for intracellular proliferation and that SIF are composed of double membrane tubules.
Mutant strains deficient in effector protein SseF or T3SS subunit SsaV display moderate or highly reduced intracellular replication,

respectively. The *sseF* strain induces only single membrane SIF, while the *ssaV* strain is unable to induce SIF formation. Here, we applied proteomic analyses of intracellular STM to elucidate the physiological consequence of normal, aberrant or absent SIF formation.
Methods: To analyze the impact of SIF formation on the intracellular lifestyle of STM, we infected RAW264.7 macrophages with various strains and isolated *Salmonella* cells between 12 to 16 h p.i. For quantitative proteomics we used LC-MSE on a Waters-Synapt G2-S HDMS to detect the changes in protein abundance between the strains.
Results: Around 1,200 STM proteins were identified and quantified for each strain, of which about 150 proteins were significantly altered in their abundance between two compared strains. In comparison to STM wild type, both mutant strains showed reduced protein biosynthesis, suggesting an overall attenuated metabolism. Furthermore, proteins involved in cell defense were up-regulated in both mutant strains. The analysis revealed that the *sseF* mutant strain showed an increased capacity in uptake of iron, as well as of amino acid, suggesting an increased metabolic activity compared to SsaV-deficient strain. Moreover, these requirements indicate a limitation of amino acids and metals inside single membrane SIF in contrast to double membrane SIF induced by wild type STM.
Conclusion: Proteomics of intracellular STM SPI2 mutant strains provide insights into the role of SIF for the intracellular survival and proliferation. Changes in the metabolism of the *sseF* mutant strain compared to STM wild type and the *ssaV* mutant strain indicate an altered nutritional supply inside the in single membrane SIF compared to double membrane SIFs or SCV. Further proteome profiles of other mutant strains with defects in SIF biogenesis will help to elucidate the function of SIF on STM pathogenesis.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

776/MPP
VraH of *Staphylococcus aureus* contributes to AMP resistance and pathogenicity
P. Popella*[1], S. Krauss[1], P. Ebner[1], M. Nega[1], F. Götz[1]
[1]*University, Mikrobielle Genetik, Tübingen, Germany*

Staphylococcus aureus as a major cause of a variety of infectious diseases in humans possesses a set of up to 17 two-component systems (TCSs). Those are comprised of a membrane-bound sensor histidine kinase (HK), and a cognate response regulator (RR). The TCS BraSR leads to upregulation of vraDE expression that encodes an ABC transporter playing a role in bacitracin and nisin resistance. Here we show that full activity of VraDE in *S. aureus* is dependent on the small trans-membrane protein VraH that contributes to high level resistance against gallidermin and daptomycin. This system is important for *S. aureus* to cope with antimicrobial peptides (AMPs) and to survive in an infection model.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

777/MPP
The stringent response in *Neisseria meningitidis* and the role of RelA in meningococcal colonization and virulence
L. V. Hagmann*[1], B. Joseph[1], B. Conrad[1], C. Schoen[1]
[1]*Institut für Hygiene und Mikrobiologie, Würzburg, Germany*

Introduction: In numerous human pathogenic bacteria the alarmone (p)ppGpp was shown to mediate a global reprogramming of gene expression during the stringent response and to be crucial for bacterial virulence. We could recently demonstrate that *Neisseria meningitidis* codes for a functional (p)ppGpp synthetase RelA that is required for meningococcal survival in human whole-blood in a strain-dependent manner.
Objectives: Here, we report the first transcriptome analysis of the stringent response regulon in a ß-proteobacterium and assess the impact of RelA on meningococcal invasiveness to better understand the condition-dependent role of the stringent response for meningococcal physiology and virulence.
Materials & Methods: Growth of the serogroup B strain MC58 and of a Δ*relA* mutant strain was tested in meningococcal minimal medium with and without the addition of 20 amino acids. The transcriptome in the MC58 wild-type and the Δ*relA* mutant strain was analyzed in rich medium and after addition of 100 µg/ml DL-serine hydroxamate (SHX) using oligonucleotide microarrays. Static biofilm formation was analyzed in a semi-defined medium, and cell invasion and adhesion experiments were performed using the human nasopharyngeal cell line Detroit 562.
Results: Growth experiments showed that RelA is required for meningococcal growth in the absence of external amino acids. Whereas we could not detect any significant transcriptome differences between the wild-type and the Δ*relA* mutant strain in the early mid logarithmic growth phase, over 400 genes were differentially expressed between both strains in the late logarithmic growth phase, mainly for genes involved in energy production and conversion as well as for genes involved in replication, recombination and repair. SHX-induced amino acid starvation in the early mid logarithmic growth phase resulted in an immediate growth reduction in strain MC58 following the addition of SHX and a rapid change in gene expression comprising over 200 genes which were enriched for cell envelope functions. Four hours after addition of SHX in the late logarithmic growth phase there was yet no overrepresentation of any functional class among the 220 differentially expressed genes. Furthermore, whereas there were no differences in static biofilm formation and cell adhesion between the Δ*relA* mutant and the wild-type strain, the deletion of *relA* resulted in a decreased invasion rate.
Conclusion: Our data indicate a complex regulatory interplay between the stringent response pathway and amino acid metabolism involving *relA* and suggest that *relA* might be dispensable for colonization but not cell invasion in *N. meningitidis*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

778/MPP
Adaptation mechanisms of *Actinobacillus pleuropneumoniae* to its persistent life form in swine
J. Frömbling*[1], E. L. Sassu[2], J. Spergser[1], T. Grunert[1], I. Hennig-Pauka[3], M. Ehling-Schulz[1]
[1]*Institute of Microbiology, Vetmeduni Vienna, Department of Pathobiology, Vienna, Austria*
[2]*Institute of Immunology, Vetmeduni Vienna, Department of Pathobiology, Vienna, Austria*
[3]*Clinic for Swine, Vetmeduni Vienna, Department for Farm Animals and Veterinary Public Health, Vienna, Austria*

Actinobacillus pleuropneumoniae, the causative agent of porcine pleuropneumonia causes significant losses in the pig industry. Several studies addressed the understanding of acute infection but little is known about chronic infections. For gaining insights into the *A. pleuropneumoniae* adaptations mechanisms to its host, an acute and a chronic pig infection trail was carried out. *A. pleuropneumoniae* was re-isolated from lungs, tonsils and noses and subjected to Fourier-Transform Infrared (FTIR-) Spectroscopy. Spectral analysis revealed remarkable differences within the bacterial metabolic fingerprints. Chemoimetric analysis showed that the isolates from both, the acute and the chronic trial, are grouped in distinct clusters, one containing the nose and tonsil isolates and one comprising lung isolates. Major differences were

found within the carbohydrate and protein composition. Preliminary crude capsule analyses via FTIR indicate that differences within the carbohydrate region are partly associated with capsule thickness and composition. These data indicate a possible shift of the isolates from noses and tonsils towards the expression of cell adherence factors as well as distinct metabolic adaptation of *A. pleuropneumoniae* to the porcine upper respiratory tract already during early infection. FTIR data as well as preliminary data of serological proteome analysis (SERPA) are fostering our hypothesis that rapid physiological adaptation of the bacteria to the host paves the way for chronic *A. pleuropneumoniae* infections.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

779/MPP

The role of Salmonella Pathogenicity Island 2 (SPI2) in the course of neonatal *Salmonella* infections

K. van Vorst[1,2], A. Dupont[3], K. Zhang[3], C. Pfarrer[4], U. Repnik[5], G. Griffiths[5], M. Hensel[6], P. Valentin-Weigand[2], M. Hornef[3], M. Fulde*[1]
[1]*Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*
[2]*University of Veterinary Medicine Hannover, Institute of Microbiology, Hannover, Germany*
[3]*RWTH University Hospital, Institute of Medical Microbiology, Aachen, Germany*
[4]*University of Veterinary Medicine Hannover, Department of Anatomy, Hannover, Germany*
[5]*University of Oslo, Department of Biosciences, Oslo, Norway*
[6]*University of Osnabrück, Division of Microbiology, Osnabrück, Germany*

Non-typhoidal *Salmonella* (NTS) are among the most prevalent causative agents of infectious diarrheal disease in humans worldwide but also contribute to invasive infections in infants. The pathogenicity of *Salmonella* is conferred by horizontally acquired chromosomal regions, called Salmonella pathogenicity islands (SPIs). SPI1 and SPI2 encode type III secretion systems (T3SS) that translocate sets of effector proteins into the host cell cytosol. The role of SPI2-T3SS effectors in host-pathogen interactions has been extensively studied *in vitro* and it is widely accepted that SPI2 is crucial for the establishment of an intracellular compartment, the *Salmonella* containing vacuole (SCV), which allows the bacteria to survive and replicate inside the host cell. In the present study, we used our newly established neonatal mouse model to extend our knowledge on the role of individual SPI2-T3SS effector proteins in the establishment and progression of systemic *Salmonella* infections. Oral infection of neonate mice with wild-type and SPI2-deficient *Salmonella* resulted in a quantitatively similar colonization of the gastrointestinal tract, but significantly reduced numbers of SPI2 mutants in systemic organs, such as liver and spleen. In contrast to the role of SPI2 as prerequisite for SCV formation *in vitro*, mutants were able to establish and maintain intraepithelial SCVs *in vivo*. In fact, SPI2-deficient bacteria grew to high numbers inside SCVs without harming their respective host cell. By evaluating in total 15 isogenic *Salmonella* strains deficient in SPI2 effector proteins, we demonstrate that SifA, a SPI2 effector, which is anchored to the SCV´s membrane, significantly contributes to the SPI2-dependent phenotype *in vivo*. Our results suggest that its lack prevents SCV transmigration from the apical to the basolateral site of the neonate enterocytes and, finally, systemic spread of *Salmonella in vivo*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

780/MPP

Screening of cell line infectivity among various *Salmonella* isolates of human, cattle, swine and chicken origin

M. Burdukiewicz[1], A. Mikołajczyk[2], I. Szabo[3], A. Böhm[4], J. Nitschke[4], P. Schierack[4], R. Kolenda*[4]
[1]*University of Wrocław, Department of Genomics, Wrocław, Poland*
[2]*Wrocław University of Environmental and Life Sciences, Department of Biochemistry, Pharmacology and Toxicology, Wrocław, Poland*
[3]*Federal Institute for Risk Assessment (BfR), National Salmonella Reference Laboratory, Berlin, Germany*
[4]*Brandenburg University of Technology Cottbus-Senftenberg, Institute of Biotechnology, Senftenberg, Germany*

Introduction: Adhesion and invasion to epithelial cells is one of the first steps during the pathogenesis of *Salmonella* infections. *Salmonella* has evolved various strategies to adhesion and invasion and different serovars have developed a specific combination of different adhesins. To study these interactions in vitro adhesion/invasion models are often in use. The reference assay to determine the number of bacteria adhering/infecting cell lines is the colony forming unit (CFU) determination by plating serial dilutions of bacteria suspensions on LB agar.
Objectives: Currently used methods are not suitable for large scale studies of *Salmonella* cell line infectivity. Therefore, our aim was to develop an automated microscopy-based system for bacterial infection assays.
Materials & methods: *Salmonella* Typhimurium (Tym) SL1344 GFP was used as a standard strain in infection assays on three cell lines- IPEC-J2 (porcine), Caco-2 (human) and CHIC8-E11 (avian). To check the linear range of assays various dilutions of bacteria were used (2x104-3x108). The bacteria were incubated with cells for 1h, unbound bacteria were washed, cells and bound bacteria were fixed with PFA and nuclei were stained with DAPI. Automated bacteria counting was done with an automated fluorescence microscope (VideoScan). For screening, 127 *Salmonella* strains from five serotypes of human, cattle, swine and chicken origin were transformed with the pFPV25.1Kan plasmid and used in infection assays for 1h and 4h on cell lines, counted with the VideoScan, and fitted to the appropriate statistical model.
Results: The quantification of *Salmonella* was possible with wide range of bacteria dilutions. The linear range of assay was 2x104-2.5x107 (R2=0.96, maximum of 14.000 bacteria/mm2), 2x104-3x107 (R2=0.96, maximum of 10.400 bacteria/mm2) and 2x104-3x107 (R2=0.92, maximum of 12.350 bacteria/mm2) for respectively IPEC-J2, Caco-2 and CHIC8-E11. In screening of 127 *Salmonella* isolates median infection rate was the highest for CHIC8-E11 and the lowest for IPEC-J2. Among all tested groups on all cell lines, S. Gallinarum had the lowest infections rate on CHIC8-E11 and S. Choleraesuis had the highest infections rate on Caco-2. Overall, S. Gallinarum displayed the lowest infection rates regardless of the incubation time. S. Choleraesuis had the largest median absolute increase of bacteria between incubation times (ΔInf=Inf4h-Inf1h) on Caco-2. On CHIC8-E11 ΔInf was the highest for S. Dublin and on IPEC-J2 for S. Typhimurium isolated from humans. Nearly all *Salmonella* had the lowest ΔInf on IPEC-J2 with the exception for of S. Gallinarum, which surprisingly had the highest ΔInf on this cell line.
Conclusion: The new VideoScan module enables quantification of bacteria in a wide range of bacterial starting inoculum. Analysis of a preliminary screen with VideoScan provides valuable information that needs further investigation.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

781/MPP

Analyzing brush border effacement during invasion of *Salmonella* Typhimurium in polarized epithelial cells by correlative light and electron microscopy

C. Kommnick*[1]
[1]*University of Osnabrueck, FB05 Microbiologie, Osnabrueck, Germany*

Question: *Salmonella enterica* sv. Typhimurium (STM) is a facultative intracellular pathogen causing self-limiting gastroenteritis after ingestion of contaminated food. Prior invasion of epithelial cells of the intestine, tight adhesion is mediated by the giant adhesion SiiE. Next the host cell actin cytoskeleton is remodeled by effector proteins of the type III secretion system (T3SS) encoded by *Salmonella* pathogenicity island 1 (SPI1). We observed that interaction of STM with polarized epithelial cells also results in transient effacement of the apical brush border.
Known to manipulate the reformation of the F-actin cytoskeleton in host cells, SopE SPI1-T3SS effector protein is a major factor resulting in effacement of the brush border and uptake of STM by macropinocytosis. Due to accumulation of F-actin on the contact side of STM invasion, the formation of membrane ruffles is a distinct morphologic feature, which needs to get elucidated. Thus, we aim for understanding the molecular and cellular mechanisms of the brush border effacement by analyzing dynamics as well as the ultrastructure.
Methods: To obtain ultrastructural details of the membrane ruffle morphology during infection by STM SL1344 (SopE positive) and NCTC12023 (naturally lacking SopE), scanning electron microscopy (SEM) was performed. Additionally, Protein A immuno-gold labelling of SiiE was achieved to visualize adherence to microvilli. As invasion is a fast and dynamic process, lasting only 5 to 7 minutes from first contact of STM to completed uptake into epithelial cells and restoration of microvilli, each event needs to get correlated individually by time. To define the morphologic stages of membrane ruffles, a correlative light and electron microscopy (CLEM) approach using spinning disk confocal microscopy (SD) and SEM was devised. Therefore, polarized epithelial cells MDCK Lifeact-meGFP and mCherry-expressing STM were used.
Results: We were able to label SiiE with Protein A gold, visualizing clusters on the bacterial surface and secreted proteins adhering to microvilli. There, we observed that NCTC12023 induces smaller bulky membrane ruffles whereas SL1344 induces larger apical remodeling, indicating stringer impact on brush border effacement. Further, we established a workflow for CLEM using SD and SEM, gaining our first morphometric data by observing ruffle formation over time via live cell imaging, followed by a morphology analysis on an ultrastructural level.
Conclusion: We could show that lack of certain SPI1-T3SS effector proteins e.g. SopE have an impact on ruffle formation due to a massive re-organization of F-actin. Moreover, gold-labelling of SiiE is a good choice to visualize bacterial adhesion to microvilli. Finally, our newly established CLEM approach is a promising method to clarify the morphology of membrane ruffles over the time of invasion.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

782/MPP

Pathophysiological role of the nucleoside substrate-binding protein PnrA of *Streptococcus pneumoniae*

M. Abdullah*[1], F. Jennert[1], T. Kohler[1], L. Petruschka[1], M. T. Batuecas[2], R. Molina[2], J. A. Hermoso[2], S. Hammerschmidt[1]
[1]*Interfaculty Institute for Genetics and Functional Genomics, Genetics of Microorganisms, Greifswald, Germany*
[2]*Institute of Physical-Chemistry, CSIC, Department of Crystallography and Structural Biology, Madrid, Spain*

Introduction: *Streptococcus pneumoniae* produces a large repertoire of ABC transporters to maintain bacterial fitness and virulence in its various ecological niches in humans. The *pnr* (**p**neumococcal **n**ucleoside **r**eceptor) operon is constituted of *pnrA*, the other genes of the Pnr ABC transporter and the upstream genes are annotated for nucleoside metabolism. PnrA is a pneumococcal lipoprotein and substrate-binding proteins (SBP) of the nucleoside PnrABC transporter system. Here we introduce the structure of PnrA, which is highly abundant o the surface and we further delineate its role in the pathophysiology of pneumococci.
Methods: Pneumococcal mutants deficient in PnrA (SP_0845) were generated by insertion deletion mutagenesis in pneumococci with different genetic backgrounds. Growth behaviour was tested and mutants were characterized on the molecular and protein level by PCR, immunoblot analysis, and flow cytometry. To decipher the structure of the substrate-binding protein PnrA by X-ray crystallography, PnrA was produced heterologously in *E. coli*. To study the effect of PnrA to resist phagocytosis and intracellular killing, pneumococcal uptake by murine macrophages J774 was tested. The role of PnrA in pneumococcal adherence was assessed with A549 alveolar lung epithelial cell line and *in vivo* virulence studies were conducted using an acute pneumonia and sepsis mouse infection models.
Results: Growth experiments showed no difference for the *pnrA*-mutant in the complex medium THY and chemically-defined RPMI 1640modi. medium compared to the isogenic wild-type. Flow cytometric analysis and immunoblots after protein fractionation indicated the surface localization of PnrA and release into the supernatant upon inactivation of the diacylglyceryl-transferase Lgt. Structural analysis indicated similarities to the purine receptor TmpC of *Treponema pallidum* (1). Phagocytosis experiments revealed a higher number of recovered intracellular pneumococci of the *pnrA*-mutant when compared to the wild-type. IN accordance, immunofluorescence microscopy demonstrated a higher uptake of *pnrA*-mutant bacteria, while intracellular survival was identical to the isogenic parental strain. In the acute pneumonia model mice infected with the PnrA-deficient pneumococci showed, although not significantly, improved survival, while the *pnrA*-mutant virulence was not similar to the wild-type in the sepsis model.
Conclusion: Pneumococcal PnrA, highly abundant on the cell surface, is important for pneumococcal fitness in the respiratory tract, where limitations of nutrients require intact substrate uptake systems.

References

[1] Deka, R.K., C.A. Brautigam, X.F. Yang, J.S. Blevins, M. Machius, D.R. Tomchick, and M.V. Norgard. 2006. The PnrA (Tp0319; TmpC) lipoprotein represents a new family of bacterial purine nucleoside receptor encoded within an ATP-binding cassette (ABC)-like operon in Treponema pallidum. *The Journal of biological chemistry*. 281:8072-8081

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

783/MPP

The impact of oxygen limitation on the secretome of *Pseudomonas aeruginosa*

A. Schmidt*[1], E. Schäfer[1], C. Rother[1], I. Droste-Borel[2], B. Macek[2], S. Schwarz[1]
[1]*University Hospital Tübingen, Institute for Microbiology and Infection Medicine, Tübingen, Germany*
[2]*University Tübingen, Proteome Center, Tübingen, Germany*

The leading cause of death in Cystic Fibrosis (CF) patients are persistent infections of the airways by *Pseudomonas aeruginosa* which lead to chronic inflammation and pulmonary deterioration. The viscous mucus and oxygen consumption by bacteria and host cells create hypoxic to anoxic niches in the CF lung which are found in adult but also in pediatric CF patients. This indicates that oxygen limitation shapes the interaction of *P. aeruginosa* with host cells during both acute and chronic infection. To shed light on the impact of anaerobiosis on *P. aeruginosa* proteins that likely interact with host cells, we performed a global secretome analysis. For this, we grew *P. aeruginosa* PAO1 in M9 minimal medium supplemented with 0.4% glucose, 15 mM KNO_3 and 100 mM $FeCl_3$ under defined aerobic and anaerobic conditions and analyzed the culture supernatant using label free quantitative LC-MS/MS. We identified several proteins that were secreted by PAO1 specifically in the absence of oxygen or at significantly higher levels compared with aerobic conditions in all three biological and technical replicates. Among these are for example the outer membrane porin OprE, a quorum sensing regulated protein of unknown function and a probable amino acid binding protein, all of which contain a predicted signal sequence. An example of a protein that displayed a significantly lower abundance in the supernatant of PAO1 grown under anaerobic conditions is FlgM. Importantly, the oxygen-dependent secretion of these proteins could be recapitulated in a clinical CF isolate. Immunoblot analysis of supernatants of PAO1 propagated in the presence and absence of oxygen probed with CF sera showed that the patients developed antibodies against proteins secreted under anaerobic conditions. The identity of these proteins will be determined in future studies.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

784/MPP

The human pathogenic fungus *Aspergillus fumigatus* counteracts phagolysosomal vATPase asssembly by interference with flotillin-dependent rafts through conidial DHN melanin

F. Schmidt*[1], H. Schmidt[1], A. Thywißen[1], M. Röcker[1], T. Heinekamp[1], S. G. Filler[2], A. A. Brakhage[1]
[1]*Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie e.V. - Hans-Knöll-Institut-Jena , Molekulare und Angewandte Mikrobiologie, Jena, Germany*
[2]*Los Angeles Biomedical Research Institute, Torrance, California, United States*

The filamentous fungus *Aspergillus fumigatus* is the most important airborne fungal pathogen of humans. An important virulence determinant is represented by the dihydroxynaphthalene (DHN)-melanin layer of conidia, which inhibits acidification of phagosomes. After inhalation, conidia get into contact with membranes of alveolar macrophages, which are composed of lipid rafts, *i.e.*, defined membrane domains with a distinct lipid composition and high cholesterol content. Lipid rafts form dynamic signaling platforms. The chaperon flotillin contributes to the structure of lipid rafts and thus represents a marker protein. To elucidate the role of flotillins for phagocytosis of conidia and acidification of phagolysosomes, bone marrow-derived macrophages (BMDMs) of Flot1/2 knockout mice were compared to C57BL7/6 wild-type cells. BMDMs were co-incubated with DHN-melanin-coated wild-type *A. fumigatus* conidia as well as pigmentless *pksP* mutant conidia. We found that DHN-melanin-containing conidia showed reduced phagocytosis, interacted with membranes and disturbed lipid rafts. Finally, the knockout of Flot1/2 led to reduced acidification of phagosomes due to reduced assembly of vATPase. By this mechanism, *A. fumigatus* is able to evade the attack of host phagocytes, and to generate a niche for survival and initiation of an infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

785/MPP

The heterogeneity of fibrinogen binding in *Staphylococcus lugdunensis* is due to capsular polysaccharides and a frame shift mutation in the *fbl* gene.

L. Marlinghaus*[1], C. Lemke[1], M. Mucke[1], J. Ameln[1], S. G. Gatermann[1]
[1]*Ruhr-Universität, Medizinische Mikrobiologie, Bochum, Germany*

Introduction: *Staphylococcus lugdunensis* is a commensal and integral part of the normal skin flora but also an important pathogen that causes several mild to serious infections similar to those caused by *Staphylococcus aureus*, like endocarditis, sepsis, skin and soft tissue infections. The fibrinogen binding protein clumping factor A (ClfA) is one of the key virulence factors in *S. aureus* and a criteria used to distinguish *S. aureus* from other coagulase-negative staphylococci. *S. lugdunensis* is also able to bind fibrinogen by means of a ClfA homolog, the Fbl. All *S. lugdunensis* strains possess the *fbl* gene analyzed by PCR but only about 55% of the strains bind fibrinogen.
Objectives: Analyze the influence of capsular polysaccharides and *fbl* repeat length on fibrinogen binding capacity of *S. lugdunensis* and the distribution of a truncated *fbl* gene.
Materials & methods: The fibrinogen binding capacity of capsular mutant strains was analyzed. The *fbl* repeat lengths was determined by PCR. The *fbl* genes were sequenced.
Results: The repeat region of *fbl* has a length of 300-1000 bp. In most of the strains (77.5%) the repeats were longer than 750 bp. In capsule mutants a strong increase of fibrinogen binding was observed when the repeat region had a length of 300 bp and 750 bp but not in strains with a repeat region of 850 bp and 900 bp. We partially sequenced the *fbl* of 77 non-fibrinogen binding strains and observed a frame shift mutation in 50.6% of the cases. The deletion of 4 base pair in these 39 strains is always located at position 12-15 which is a direct repeat of position 16-19. One may think this might be an on/off switch for fibrinogen binding controlled by a slipped strand mispairing mechanism. However, the distribution of a gene encoding for a beta-lactamase *blaZ* and 5 nucleotide dimorphisms upstream of *fbl* indicate that the strains carrying the truncated *fbl* may belong to one lineage. Among these strains 94% were *blaZ*-positive whereas among the strains possessing the functional *fbl* only 23.8% were *blaZ*-positive.
Conclusion: Fibrinogen binding capacity is influenced by capsular polysaccharides in strains of *S. lugdunensis* harboring an *fbl* gene with a short repeat region. In about 50% of the non-fibrinogen binding strains the *fbl* gene was truncated. These strains may be a lineage and have a very high incidence for beta-lactamase carriage.
Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

786/MPP

Analysis of *Corynebacterium diphtheriae* macrophage interaction reveals dispensability of mycolic acids and leads to the identification of a new gene involved in synthesis of the mycolic acid layer

L. Bäumer*[1], A. Burkovski[1], A. Bhatt[2], V. Sangal[3]
[1]*Lehrstuhl für Mikrobiologie, Biologie, Erlangen, Germany*
[2]*Institute of Microbiology and Infection and School of Biosciences, Birmingham, United Kingdom*
[3]*Faculty of Health and Life Sciences, Northumbria University , Newcastle, United Kingdom*

Corynebacterium diphtheriae is the causative agent of diphtheria, an upper respiratory tract illness with severe symptoms including pseudomembrane formation due to the toxin. *C. diphtheriae* is as a member of the CMNR group closely related to the genera *Mycobacterium*, *Nocardia* and *Rhodococcus.* The most prominent virulence factor of *Mycobacterium tuberculosis* is the so called cord factor, trehalose 6,6´-dimycolate (TDM). TDM is found in the cell envelope and leads to the blocking of the formation of phagolysosomes, which protects the bacteria from killing by macrophages. Based on this information, the aim of this study was to investigate three different *C. diphtheriae* strains, two mycolic acid producers and one mycolic acid free strain, with regard to their interaction with phagocytic cells and the invertebrate infection model *C. elegans*. Our results indicate that *C. diphtheriae* strains were able to slow down phagosome maturation in murine and human cell lines compared to the non-pathogenic *Corynebacterium* strain *C. glutamicum*. Additionally, NF-κB induction was analyzed in response to infection with *C. diphtheriae*, which revealed strongest activation when MOI1 was tested. Using higher MOIs results in lower activation signals, which might indicate that the bacteria have a detrimental effect on the cells.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

787/MPP

Lysozyme triggers biofilm formation of *Streptococcus gallolyticus* subsp. *gallolyticus*

I. Grimm*[1], J. Dreier[1], C. Knabbe[1], T. Vollmer[1]
[1]*Herz- und Diabeteszentrum NRW, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany*

Introduction: Streptococcus gallolyticus subsp. gallolyticus (SGG) is recognized as a pathogen in about 20% of streptococcal-caused infective endocarditis cases. Colonization and biofilm formation at the endocardium is an important virulence factor for this bacterium to survive human defend mechanisms. For a better understanding of the mechanisms of lysozyme resistance and biofilm formation of SGG, the transcriptome in presence of lysozyme was analyzed in this study.
Methods: Adhesion of five SGG strains to polysterol in presence of lysozyme (10 mg/ml and 20 mg/ml) was verified by crystal violet staining after 5 and 16 hours of incubation. For transcriptome analysis, RNA was extracted from two SGG strains in BHI medium with or without 10 mg/ml lysozyme after five hours of incubation to examine early reaction to lysozyme. The RNA was processed, Cyanine3 labeled and hybridized to microarrays. Based on four SGG genomes, the full genome microarray for SGG analysis was developed (Oaklabs, Hennigsdorf). It consists of 10,607 oligonucleotides which represent 4,382 genes. Analysis was done by Direct Array and log2 values were only considered when higher than +1 or smaller than -1 with a p-value smaller than 0.05. Microarray results were verified with relative quantitative real-time PCR.
Results: The biofilm formation of SGG is strain-dependent. The strain DSM 16831 formed more biofilm at polysterol compared to the other strains after 16 hours of incubation. It was also shown, that lysozyme leads to significantly more biofilm formation in two (BAA-2069 and LMG 17956) of five strains within five hours and of four strains within 16 hours of incubation. The quantity of the biofilm formation of the strain DSM 16831 decreased with increased lysozyme resistance after five and 16 hours of incubation compared to control without lysozyme. Lysozyme resistance and biofilm formation of the SGG isolates BAA-2069 and UCN 34 was examined by transcriptome analysis with full genome microarrays. It was shown that gene expression of the dlt operon was upregulated in presence of lysozyme as well as genes whose products are involved in transcription and translation, DNA repair and hydrogen peroxide resistance. The gene expression of the microcin immunity protein (mccF) and competence induced protein A (cinA) which belong to bacterial immunity were also increased. Expression of genes which belong to bacterial competence, acid tolerance and antibiotic resistance were decreased.
Conclusion: D-alanylation (dlt operon) is increased in presence of lysozyme which leads to resistance to the cationic microbial peptide activity of this enzyme. Additionally, this study showed for the first time that lysozyme triggers biofilm formation in a bacterial species which could be a benefit for the pathogenesis of SGG. The proteins cinA and mccF in SGG whose expression is induced in presence of lysozyme may lead to this rapid biofilm formation.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

788/MPP

Intrinsically unstructured PE/PPE family proteins of *Mycobacterium tuberculosis*: Evidence of role in pathogenesis *via* molecular mimicry

J. Ahmad*[1,2], A. Farhana[3], R. Pancsa[4], A. Srinivasan[2], A. K. Tyagi[5], M. M. Babu[4], N. Z. Ehtesham[1], S. E. Hasnain[6,7,8]
[1]*National Institute of Pathology, Inflammation Biology and Cell Signalling Laboratory, New Delhi, India*
[2]*All India Institute of Medical Sciences, Department of Biophysics, New Delhi, India*
[3]*University of Alabama, Alabama, United States*
[4]*Medical Research Council, Laboratory of Molecular Biology, Cambridge, United Kingdom*
[5]*Universty of Delhi South Campus, Department of Biochemistry, New Delhi, India*
[6]*JH Institute of Molecular Medicine, Jamia Hamdared University, New Delhi, India*
[7]*Indian Institute of Technology, Molecular Infection and Functional Biology Laboratory, New Delhi, India*
[8]*University of Hyderabad Campus, Dr. Reddy's Institute of Life Sciences, Hyderabad, India*

Pathogens use linear motifs and intrinsically disordered regions within a protein to perturb and hijack the host cell networks for a successful and productive infection. *Mycobacterium tuberculosis* (*M.tb*), the causal organism for tuberculosis (TB) that takes one human life every 15-20 seconds globally, harbors the highest percentage (14.5%) of intrinsically disordered proteins (IDPs) among bacterial and archeal genomes. We investigated the role of IDP-rich PE/PPE/PGRS protein family of *M.tb* in survival and pathogenesis. Employing computational approaches we searched for disordered protein regions in PE PPE, Mce, MmpL families which have known roles for pathogenesis of *Mycobacterium tuberculosis*. PE_PGRS subfamily displayed unusually high levels of disordered/partially-disordered stretches compared to any other family in the proteome and was highly enriched in average number of ANCHOR binding sites, eukaryotic linear motifs (ELMs) and has highly biased amino acid composition rich in disorder promoting alanine and glycine residues. The functional implications of these *in-silico* results were investigated using a member of PE-PPE family as an example. This protein was found to have an iron-binding N-terminal ordered region followed by

two trans-membrane domain, a disordered C-terminal segment harboring number of ELMs and eukaryotic nuclear localization signal (NLS). The expressed protein was associated with mycobacterial cell membrane and cleaved by *M.tb* specific factors into N-terminal segment and an unstructured C-terminal segment with two different functions, creating tolerogenic DCs and in dissemination.
Our study provides evidence supporting the role of intrinsically disordered stretch within a PPE protein in performing contrasting functions to modulate the host processes possibly through molecular mimicry and cross-talk in two spatially distinct host environments so as to benefit *M.tb* in terms of survival and pathogenesis.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

789/MPP

Functional interaction between Mycobacterium tuberculosis RipA, an invasion associated secretory protein, and MoxR1

M. Bhuwan*[1,2], S. E. Hasnain[2,3,4], N. Z. Ehtesham[1]
[1]*National Institute of Pathology, Inflammation Biology and Cell Signaling, New Delhi, India*
[2]*Indian Institute of Technology, New Delhi, Molecular Infection and Functional Biology Laboratory, Kusuma School of Biological Sciences, New Delhi, India*
[3]*Jamia Hamdard University , JH-Institute of Molecular Medicine, New Delhi, India*
[4]*University of Hyderabad, Dr. Reddy's Institute of Life Sciences, Hyderabad, India*

Mycobacterium tuberculosis (*M.tb*) is a leading cause of death worldwide. The Tat (twin-arginine translocation) protein secretion system is present at the cytoplasmic membrane of mycobacterium and is known to transport folded proteins and is reported to be essential for many of the important bacterial processes that include cell wall biosynthesis. RipA, a secretion and invasion protein of *M.tb* has endopeptidase activity. The MoxR1 protein belongs to ATPases family and is associated with various cellular activities. RipA and MoxR1 were expressed and purified to homogeneity from *E.coli*. Recombinant MoxR1 protein was characterized in terms of its biophysical feature. Protein aggregation assay of recombinant MoxR1 at higher temperature showed resistance to aggregation. Furthermore, recombinant MoxR1 was able to prevent the aggregation of Maltodextrin glucosidase (MalZ) protein at higher temperature which is enhanced in the presence of ATP suggesting its role as a chaperone. Role of MoxR1 was also demonstrated in *in vivo* folding of RipA in *E. coli* cells. *In-silico* analyses pointed to a protein-protein interaction between *M.tb* RipA and MoxR1 as well as RipA and Mce2B (mammalian cell entry protein). Furthermore, using bimolecular fluorescence complementation (BiFC) assay the interaction of these proteins was confirmed in HEK293T using confocal microscopy. It has been shown earlier that recruitment of the autophagy protein to sites of bacterial entry leads to lysosomal degradation of the invading bacteria. Our bioinformatics analysis of RipA protein using eukaryotic linear motif resources (www.elm.eu.org) showed the presence of canonical LIR motif that binds to Atg8/LC3 protein family members to mediate processes involved in autophagy. The endopeptidase activity of RipA protein will degrade the cell- wall material of *M.tuberculosis* and the bacterial breakdown products might then be recognized by the pattern recognition receptor to activate the inflammatory responses within the host cell. Overall our study demonstrates *M.tb* MoxR1 as novel molecular chaperone involved in maturation or refolding of specific protein complexes and the secretion of RipA protein with likely role in pathogenesis.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

790/MPP

***Mycobacterium tuberculosis* Signature Proteins: Characterisation and Immune Validation as a Novel Vaccine Candidate**

J. Ahmad[1], J. A. Sheikh*[1], S. A. Rahman[2], M. P.[1], S. K. Arora[1], S. E. Hasnain[3,4], N. Z. Ehtesham[1]
[1]*National Institute of Pathology, Inflammation Biology and Cell Signaling Laboratory, New Delhi, India*
[2]*European Molecular Biology Laboratory, European Bioinformatics Institute, Hinxton, United Kingdom*
[3]*Indian Institute of Technology, Molecular Infection and Functional Biology Laboratory, New Delhi, India*
[4]*Jamia Hamdard, Institute of Molecular Medicine, New Delhi, India*

To combat intracellular bacterial infections, one of the important mechanism developed by host machinery is the production of antibacterial molecules like reactive oxygen and nitrogen species. *Mycobacterium tuberculosis* portrays a classical paradigm of persistent bacterial infections. Therefore, hijacking the host machinery and combating the host stress reponses is key for its surivival inside macrophages. We describe the identification and characterization of proteins that are exclusively present in *M.tuberculosis* and elucidated their role in immune modulation. We show their utility as diagnostic biomarkers apart from their role as pathogen specific virulence factors. Exploiting *in-silico* comparative genomic and proteomic approaches we first identified the unique proteins of pathogenic *M.tuberculosis,* characterised the various binding activities, validated the same by Fluorescence spectroscopy and conducted immunological studies in animals. One such protein, termed as signature protein, was found to be S-adenosyl dependent DNA methyltransferase and binds DNA non-specifically and protect DNA from oxidative stress by scavanging iron thereby, preventing generation of free radicals and also by physically binding DNA and providing a physical barrier. Upon immunization this protein generated Th1 response along with a multitude of polyfunctional T cells. This protein was also able to enhance the effector memory response and thus could be exploited as a potential novel vaccine candidate against tuberculosis. This signature protein being essential and having protective role may be central to *M.tuberculosis* pathogenesis in addition to its use as a specific serodiagnostic as well as DNA based TB biomarker as evident from pilot scale clinical validation studies.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

791/MPP

Analyses of methyltransferases across the pathogenicity spectrum of different mycobacterial species point to an extremophile connection.

S. Ali*[1], S. Grover[1], P. Gupta[1], P. S. Kahlon[1], S. Goyal[2], A. Grover[2], K. Dalal[1], N. Z. Ehtesham[3], S. E. Hasnain[1,4]
[1]*Indian Institute of Technology, Kusuma School of Biological Sciences, Delhi, India*
[2]*Jawaharlal Nehru University, School of Biotechnology, New Delhi, India*
[3]*Safdarjang Hospital Campus,, National Institute of Pathology, New Delhi, India*
[4]*University of Hyderabad Campus, Dr Reddy's Institute of Life Sciences , Hyderabad, India*

Tuberculosis is a devastating disease, taking one human life every 20 seconds globally. We hypothesize that professional pathogens such as *M.tb* have acquired specific features that might assist in causing infection, persistence and transmissible pathology in their host. We have identified 121 methyltransferases (MTases) in the *M.tb* proteome, which use a variety of substrates - DNA, RNA, protein, intermediates of mycolic acid biosynthesis and other fatty

acids - that are involved in cellular maintenance within the host. A comparative analysis of the proteome of the virulent strain H37Rv and the avirulent strain H37Ra identified 3 MTases, which displayed significant variations in terms of N-terminal extension/deletion and point mutations, possibly impacting various physicochemical properties. The cross-proteomic comparison of MTases of *M.tb* H37Rv with 15 different Mycobacterium species revealed the acquisition of novel MTases in MTB complex as a function of evolution. Phylogenetic analysis revealed that these newly acquired MTases showed common roots with certain extremophiles such as halophilic and acidophilic organisms. Our results establish an evolutionary relationship of *M.tb* with halotolerant organisms and also the role of MTases of *M.tb* in withstanding the host osmotic stress, thereby pointing to their likely role in pathogenesis, virulence and niche adaptation.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

792/MPP

Characterization of hypothetical Methyltransferases of *Mycobacterium tuberculosis* and analysis of their role in virulence.

S. Ali*[1], A. Ehtram[1], N. Z. Ehtesham[2], S. E. Hasnain[1,3,4]
[1]*Indian Institute of Technology, Kusuma School of Biological Sciences, Delhi, India*
[2]*Safdarjang Hospital Campus, National Institute of Pathology, New Delhi, India*
[3]*Jamia Hamdard (Hamdard University), Jamia Hamdard-Institute of Molecular Medicine Hamdard Nagar , New Delhi, India*
[4]*University of Hyderabad Campus, Prof C R Rao Road, Dr Reddy's Institute of Life Sciences , Hyderabad, India*

Tuberculosis is a major cause of mortality and morbidity worldwide. *Mycobacterium tuberculosis (Mtb)*, an etiological agent of TB, which is an intracellular pathogen capable of survival and replication in human macrophages. *Mtb*-induced modulations may affect the host cell by either activation or suppression of key immune proteins involved in immune response or pathogen persistence. Till date several virulent molecules have been shown to participate in the *M.tb* incurred disease, where they can either function to deteriorate host resistance or act as regulatory molecules that may exacerbate the infection. Post-transcriptional modifications of proteins serve a variety of purposes, from stabilizing the structure to preserving its functional integrity. Pathogenicity and post translational modifications complements each other in infections. Cells generally add methyl groups to their counterparts either DNA or protein to surpass additional information. Out of the four highly antigenic methyltransferases (MTases), Rv1523 which is exclusively present in the M.tb complex, qualifies to be the perfect antigenic protein as it also has a signal sequence which may aid in pathogenicity of *M.tb*. The string network analysis depicts the role of Rv0208c and Rv1523 in the pathogen survival as it is found to regulate the transmembrane transport protein mmpL3 and mmpL12 respectively. These two distinct MTases predicted to be involved in immunomodulation of the host as well as *Mtb* persistence, thereby enabling the mycobacteria to perpetuate its virulence. Thus this article supports the novel probable insight into the role of MTases as a pathogenicity factor rather than just being a post-translational/epigenetic protein.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

793/MPP

No evidence for a bovine mammary *E. coli* pathotype

A. Leimbach*[1,2], A. Poehlein[2], J. Vollmers[3], R. Daniel[2], U. Dobrindt[1]
[1]*Münster University Hospital (UKM), Institute of Hygiene - Microbial Genome Plasticity, Münster, Germany*
[2]*Georg-August-University Göttingen, Institute of Microbiology and Genetics - Göttingen Genomics Laboratory (G2L), Göttingen, Germany*
[3]*Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*

Bovine intramammary infections with *Escherichia coli* cost the European dairy industry approximately €2 billion per year. Pathogenic *E. coli* are traditionally classified into different pathotypes harboring specific virulence factor sets and causing a certain disease pathology. Although a mammary pathogenic *E. coli* pathotype has been proposed [Shpigel *et al.*, 2008], no common virulence factors could be determined despite extensive research efforts. Thus, environmental factors as well as the cows' innate immune response have been implicated as the sole parameters influencing mastitis outcome.
Commensal *E. coli* from the bovine habitat have been neglected in studies on *E. coli* associated with mastitis. But, virulence factors can only be defined by comparing mastitis to commensal isolates. Gene content comparisons of these two isolate groups allowed us to evaluate virulence determinants in relation to phylogenetic background.
We sequenced the genomes of eight mastitis-associated *E. coli* and six fecal commensals from udder-healthy cows [Leimbach *et al.*, 2015; Leimbach *et al.*, 2016]. Their phylogenetic history and gene content were determined in addition to eleven reference bovine *E. coli*. Additionally, the presence of well-known virulence factors was evaluated.
Both mastitis-associated and commensal strains could not be unambiguously discriminated by their phylogenetic background and the presence of virulence-associated genes. No virulence factors were significantly enriched in mastitis or commensal isolates. Instead gene content is greatly affected by phylogenetic background.
E. coli strains from bovine mastitis mirror the phylogenetic and genotypic diversity of isolates from the gastrointestinal tract. There is no evidence for a mammary gland specific pathotype. Instead, putative virulence factors should be considered fitness factors for gastrointestinal colonization. In summary, mastitis-associated *E. coli* are facultative pathogens which are recruited from the normal intestinal microbiota.

References

[1] Shpigel NY, Elazar S, Rosenshine I. 2008. Mammary pathogenic *Escherichia coli*. Curr Opin Microbiol. 11(1):60-65.
[2] Leimbach A, Poehlein A, Witten A, Scheutz F, Schukken Y, Daniel R, Dobrindt U. 2015. Complete genome sequences of *Escherichia coli* strains 1303 and ECC-1470 isolated from bovine mastitis. Genome Announc. 3(2):e00182-15.
[3] Leimbach A, Poehlein A, Witten A, Wellnitz O, Shpigel N, Petzl W, Zerbe H, Daniel R, Dobrindt U. 2016. Whole-genome draft sequences of six commensal fecal and six mastitis-associated *Escherichia coli* strains of bovine origin. Genome Announc. 4(4):e00753-16.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

794/MPP

Characterization and clonal association of an endemic MDR plasmid of *S.* Infantis in Hungary

A. Szmolka*[1], M. Szabó[2], J. Kiss[2], J. Pászti[3], F. Olasz[2], B. Nagy[1]
[1]*Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
[2]*NARIC Agricultural Biotechnology Institute, Gödöllő, Hungary*
[3]*National Center for Epidemiology, Budapest, Hungary*

Salmonella Infantis became endemic in broiler flocks in Hungary where the PFGE clone B, carrying a large multiresistant (MDR) plasmid designated as pSI54/04 emerged among isolates from poultry and man.
We hypothesised that plasmid pSI54/04 assisted dissemination of *S*. Infantis, however it is uncertain whether this spreading can be related to pSI54/04 and/or to virulence determinants residing on *Salmonella* pathogenicity islands (SPIs).
To test *in vitro* and *in vivo* pathogenic significance of pSI54/04 and of SPIs we produced plasmidic transconjugant of the plasmid-free pre-emergent strain SI69/94 by transferring pSI54/04 from the emerging MDR strain SI54/04. The impact of SPIs was tested on ΔSPI mutants of SI69/94. The above wild strains and mutants have been tested on chicken embryo fibroblasts (CEFs) and in orally infected day old chicks.
In these systems the pSI54/04 did not increase pathogenic potential of the pre-emergent strain SI69/94, while the deletion of SPIs resulted in a significantly reduced invasiveness for CEFs. The effect was more pronounced for SPI1. Characterization of pSI54/04 based on whole genome sequence data of the emerging strain SI54/04 indicated a size of ~277 kb and a high sequence similarity with the megaplasmid pESI of *S*. Infantis predominant in Israel. Based on the plasmid sequences a PCR panel was designed to identify specific regions of pSI54/04. Molecular characterization of 78 representative isolates of broiler and human origin revealed novel plasmid associations including the prototype pSI54/04 or variants of it within the emerging *S.* Infantis clone B. Such co-existence of the prototype plasmid pSI54/04 with the *bla*$_{\text{TEM-1}}$-bearing plasmid showing the highest homology with pYU39 from *S*. Typhimurium was identified in a novel multiplasmidic isolate.
Overall our present results suggest that the pathogenicity of *S*. Infantis for broilers may be less influenced by the plasmid pSI54/04 as compared to SPI1. Both factors however could contribute to the host adaptation of *S*. Infantis and the modulation of the host response concerning expression of citokines and lymphokines participating in early immune response of chicks to *S*. Infantis, and potentially allowing long time persistence. Furthermore the association of *S*. Infantis with pSI54/04 and other plasmids conferring MDR resistance may also assist the emergence and spread.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

795/MPP

Peptides inhibitors targeting the *Neisseria gonorrhoeae* pivotal anaerobic respiration factor, AniA, for the development of alternative treatments against gonorrhea

A. Sikora*[1], R. Mills[1], J. Weber[1], B. Passow[1], A. Hamza[2], A. Romaine[1], Z. Williamson[2], R. Reed[2], R. Zielke[1], K. Korotkov[2]
[1]*Oregon State University, College of Pharmacy , Corvallis, Oregon, United States*
[2]*College of Medicine, University of Kentucky, Department of Molecular & Cellular Biochemistry, Lexington, Kentucky, United States*

Background: *Neisseria gonorrhoeae* (GC) causes the sexually transmitted infection, gonorrhea. Gonorrhea is highly prevalent worldwide and has a major impact on reproductive and neonatal health. The lack of a preventive vaccine and GC superbug status necessitate development of drugs with new mechanisms of action. Here we focused on the discovery of inhibitors of nitrite reductase AniA, which is a pivotal component of the GC anaerobic respiration. The anaerobic life style is an important state during disease and favored in gonococcal biofilms, which form in cervical infections. The anti-AniA antibody has been found in the sera from women with gonorrhea, demonstrating that AniA is expressed during infection. These findings underscored the potential of AniA as a novel molecular target against gonorrhea.
Methods: To facilitate the pharmacological targeting of AniA, new crystal structure of AniA has been solved and a phage-display approach was utilized. The recombinant AniA was used as bait in an affinity capture method during panning experiments with phage display libraries expressing randomized linear dodecameric peptides or heptameric peptides flanked by a pair of cysteine residues.
Results: The new structure of AniA was refined to 1.90 Å resolution. The C-terminal residues 315-323, which has not been modeled in the previous structure, form an extra beta-strand that reaches over to the neighboring subunit and engages in intersubunit beta-strand complementation. Further, our studies showed that the nitrite reductase function of AniA is critical for GC survival under anaerobic conditions as bacteria expressing AniA with altered catalytic residues D137A H280A failed to grow. Biopanning experiments led to identification of 26 unique peptides, with one of them, C7-3, identified multiple times. Evaluation of their ability to interact with AniA using ELISA and computational docking studies revealed C7-3 as the most promising inhibitor binding in close proximity to the type II copper site of the enzyme, which is responsible for the interaction with nitrite. Subsequent studies with a synthetic C7-3 confirmed a strong inhibitory effect on the AniA nitrite reductase activity. In addition, mutational analysis coupled with enzymatic assays using purified protein and intact gonococci confirmed a predicted pivotal residue in C7-3 involved in peptide-AniA interaction.
Conclusions: Phage display approaches combined with computation docking studies and mutagenesis identified novel peptide inhibitors of AniA nitrite reductase activity. We present the first peptide inhibitors of AniA, an enzyme that should be further exploited for antigonococcal drug development. The pharmacological inhibition of AniA should disable anaerobic respiration and augment the ability of existing antimicrobials to clear the pathogen.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

796/MPP

Zinc assimilation by the human fungal pathogen *Candida albicans*

D. Wilson *[1]
[1]*University of Aberdeen, Aberdeen Fungal Group, Aberdeen, United Kingdom*

The mammalian immune system has evolved sophisticated mechanisms to withhold micronutrients such as zinc from potential invaders in a process known as nutritional immunity. In spite of this defence mechanism, pathogens still thrive and cause disease. Therefore, pathogenic microbes have, in turn, evolved mechanisms to circumvent nutritional immunity. Despite the fundamental importance of this host-pathogen "tug-of-war", its underlying mechanisms, and how they can be exploited to prevent disease remain poorly understood. *Candida albicans*, normally a member of the gut microbiota, is also the dominant fungal pathogen of humans and is responsible for both superficial, as well as life-threatening disseminated infections. We are exploring how *C. albicans* secures zinc from its human host and how this essential, yet potentially toxic, cation is mobilised within the fungal cell. Cellular uptake is pH dependent and occurs via two ZIP transporters. Following internalisation, zinc is shuttled to

zincosomes and to the fungal vacuole. *C. albicans* zinc-storage capacity allows the fungus to tolerate elevated environmental zinc, as well as to grow when this micronutrient is subsequently depleted from the culture medium. We have now created gene deletion mutants of all predicted intracellular zinc transporters in *C. albicans* to explore the molecular basis of intracellular zinc storage and utilisation. Finally, we have found that perturbing zinc homeostasis via deletion of the major import transporter prohibits *in vivo* fungal proliferation in an experimental model of systemic *C. albicans* infection.
Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

797/MPP
Identification and Investigation of Drug-Resistant Yeast Species Isolated from the Skin of Patients with Acne Clinical Protests Referred to the Dermatology Clinic
A. Nasrollahi Omran*[1], A. G. Mansori[2]
[1]*Islamic Azad University, Department of Medical Mycology, Faculty of Medicine, Tonekabon, Islamic Republic Of Iran*
[2]*Tonekabon Branch Islamic Azad University, Department of Dermatology, Tonekabon, Islamic Republic Of Iran*

Background: Acne is a pathological disorder and a chronic inflammation in the Sebaceous follicles, and one of the most popular dermatology damages that has affected millions of people worldwide.
Objectives: The aim of this study is to identify *Candida* species from patients with acne and determine was their drugs susceptibility.
Methods: In this study of 70 clinical specimens from suspected skin with acne protests were collected by sterile swab and were streaked on Sabouraud Dextrose Agar containing chloramphenicol. The plates were incubated for 48 hours in c˚37. Suspected colonies were studied through microscopic examination and subsequent passage in accordance with Mycology of standard procedures and specify the type of fungal colony color in CHROM agar for the isolation of the yeast. For final approval, *Candida Sp*. Sequencing Method (ITS2, ITS1regions) was performed, and susceptibility testing was performed to review *Candida sp.* for drug-resistant isolates based on CLSI method.
Results: Of 70 clinical isolates studied, 11species of *Candida* including *C.parapsilosis*8 (72.73%), *C.krusei* 1(12.5%), *C.lusitaniae* 1(12.5%), *C.kefir*1(12.5%), and *a Trichosporon asahi* were identified and isolated. C. *parapsilosis* isolates susceptibility to various concentrations of the anti-fungal agents to isolate Cp1 study has shown that the isolated Cp8 Cp5 with *Minimum Inhibitory Concentration* 50 equal to32,0.5,0.25 and MIC90 of <64, <1, <0.5 µg/ml Fluconazole, Itraconazole and Ketoconazole were respectively resistant. Apart from the isolation of Cp1 and Cp8, which had relative strength, almost all other species of *C. parapsilosis* isolates were susceptible to these drugs.
Conclusions: As various studies have proven that in most cases bacterial agents are involved in causing acne, according to the results of this study, it can be suggested that the yeast *Candida* can be introduced as an agent in the etiology of this disease. *Candida* species isolates can also be resistant to antifungal drugs and this could be one of several causes why sequential treatment of this disease is defeated.
Keywords: Acne Vulgaris, *Candida*, Antifungal Susceptibilities, Skin

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

798/MPP
Genotypic and phenotypic characteristics and biofilm formation of human clinical *Escherichia coli* isolates
J. Schiebel[1], A. Böhm[1], J. Nitschke[1], M. Burdukiewicz[2], J. Weinreich[1], S. Rödiger[1], P. Schierack*[1]
[1]*BTU Cottbus – Senftenberg, Senftenberg, Germany*
[2]*University of Wrocław, Wrocław, Poland*

Introduction: Bacterial biofilm formation is a widespread phenomenon and a complex process requiring a set of genes facilitating the initial adhesion, maturation, production of extracellular polymeric substances and subsequent dispersal of bacteria. Our knowledge of *Escherichia coli* (*E. coli*) biofilm formation is based on investigations of non-pathogenic *E. coli* K-12 strains. Due to the extensive focus on laboratory strains in most studies, there is poor information regarding biofilm formation by pathogenic *E. coli* isolates.
Objectives: We investigated human clinical *E. coli* isolates to verify if the occurrence of biofilm-associated genes enables them to exhibit increased biofilm formation. Furthermore, we wanted to examine if the ability of biofilm formation is a defined characteristic for certain *E. coli* pathotypes.
Materials and Methods: We enhanced our previous published VideoScan technology [Rödiger et al. 2013], which is based on fully automated fluorescence microscopy to perform high-throughput screening of biofilms. We analysed 187 *E. coli* strains for *in vitro* biofilm formation using different media in a 96 well plate format. Using the SYTO 9 staining and the VideoScan technology fluorescence intensities of the biofilms were measured and overview images from each well were taken. Furthermore we examined motility and performed PCR for biofilm-associated genes. Curli and cellulose production were analysed using Congo red and calcofluor agar method.
Results: Biofilm-associated genes were found in varying frequencies in all eight pathotypes tested (e.g. *bcsA* 96,3%, *csgA* 99,5%). In total 74,3 % of all strains are motile. The Congo red assay revealed 27,8% of rdar, 32,6% of bdar and 39,6% of saw morphotype while incubating the strains at 28°C. At 37°C only half as much of the strains (14,4%) produced the rdar morphotype. In total curli expression was less prevalent, when incubating the strains at higher temperatures, what is in agreement with the fact, that curli expression is a temperature regulated process. However, we could not find a significant correlation between genotype or phenotype and the biofilm formation of the different pathotypes. With our VideoScan technology we could identify strong biofilm formers belonging to the group of EAEC. These results could be confirmed by using crystal violet assay.
Conclusions: Our results show that the ability to form biofilms is rather correlated with the pathotype than with the genotype or phenotype of a strain. The biofilm of EAEC constituted a unique formation of bacterial communities suggesting the contribution of additional, so far unknown, factors in the process of biofilm formation, supporting their survival and virulence in host. With the VideoScan platform as an image-based technique we could detect a considerable number of biofilm formers out of 187 *E. coli* strains with EAEC being the pathotype exhibiting the strongest biofilm formers.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

799/MPP

Bacterial ligase SopA ubiquitinates and degrades host ligases TRIM56 and TRIM65 to regulate pro-inflammatory pathways during Salmonella infection

E. Fiškin**[1], S. Bhogaraju*[1,2], L. Herhaus[1], M. Hahn[1], I. Dikic[1,2,3]
[1]*Goethe University School of Medicine, Institute of Biochemistry II, Frankfurt am Main, Germany*
[2]*Goethe University, Buchmann Institute for Molecular Life Sciences, Frankfurt am Main, Germany*
[3]*University of Split, School of Medicine, Department of Immunology and Medical Genetics, Split, Croatia*

The hallmark of infections with enteroinvasive *Salmonella* Typhimurium is the induction of severe mucosal inflammation leading to gastroenteritis. *Salmonella* actively secretes a number of virulence factors including the bacterial HECT-like E3 ligase SopA into host cells to stimulate the expression of cytokines, which ultimately initiate intestinal inflammation. Here we demonstrate that SopA stimulates this inflammatory response by targeting the host RING ligases TRIM56 and TRIM65. Structural, proteomic and biochemical evidence suggests that SopA inhibits TRIM56 and TRIM65 via a dual mechanism. SopA binding to the TRIM56/65 RING domain impedes E2 recruitment and reduces catalytic TRIM ligase activity, while SopA HECT domain mediates ubiquitination of TRIM56 and TRIM65 resulting in their proteasomal degradation. *In vivo* studies with wild type, delta SopA and complemented SopA *Salmonella* strains indicate that SopA-driven degradation of TRIM56 and TRIM65 contributes to the induction of a pro-inflammatory state upon *Salmonella* infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Infection Epidemiology and Population Genetics (FG MS)

800/MSP

Molecular epidemiological analyses of rabies lyssavirus full genome sequences by means of phylogenetic methods in combination with affinity propagation clustering.

S. Fischer*[1], T. Homeier-Bachmann[1], F. J. Conraths[1]
[1]*Friedrich-Loeffler-Institut, Institut für Epidemiologie, Insel Riems, Germany*

Introduction: Rabies is one of the oldest known zoonosis, caused by rabies lyssavirus (RABV) an important species of the genus Lyssavirus. Annually, about 55.000 human deaths caused by an infection with rabies lyssavirus are counted. The virus belongs to the family of Rhabdoviridae and is a non-segmented and negative-polar RNA-virus. Its genome is 12 kb in size and codes for five viral proteins.
Objectives: Next generation sequencing enables us to generate a huge amount of RABV sequences, including full genome sequences. However, phylogenetic analysis of large datasets results in highly complex dendrograms. In order to reduce the complexity sequences are aggregated to clusters. So far, these clusters were most commonly defined by visual inspection of the phylogenetic dendrograms. This could lead to different results due to a lack of criteria for cluster definition.
Materials & Methods: Therefore, we applied affinity propagation clustering to eliminate these limitations. Affinity propagation clustering is a mathematical method that uses similarity matrices, here derived from the alignment of 392 RABV full genome sequences, to allocate all included sequences into generic clusters. In addition the optimal number of clusters is calculated. Analyses were performed in R using the Package apcluster.
Results: The calculated cluster structure as well as the cluster allocation of each individual full genome strongly corresponds to its geographical origin as well as to the structure of the phylogenetically based tree. The recent finding of two main clusters of RABV, namely New World and Terrestrial cluster can be confirmed. RABV belonging to the Terrestrial cluster circulate worldwide in terrestrial mammals while viruses from the New World cluster have been exclusively isolated in the new world most frequently from bats. In total, we observed four main clusters of RABV, which can be subdivided into distinct sub clusters. Moreover, each cluster and sub cluster can be resolved geographically according to their available Meta data information.
Conclusion: We applied the AP Clustering method to validate the presence of spatially-dependent clustering of rabies lyssavirus sequences. The application of predefined and objectified cluster definition can improve the accuracy of the spatial dependent classification system for rabies lyssavirus on the bases of full genomes sequences. Furthermore, this could be a new pipeline, to be applied to other viral systems to perform molecular epidemiological studies.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

801/MSP

High variability of *Staphylococcus aureus* strains causing wound infection among street children dwelling in Mwanza city streets in Tanzania

N. Moremi*[1,2], M. Hingi[2], H. Claus[1], U. Vogel[1], S. E. Mshana[2]
[1]*Institut für Hygiene und Mikrobiologie, Wuerzburg, Germany*
[2]*Catholic University of Health and Allied Sciences, Mwanza, Tanzania*

Introduction: Epidemiology of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has shown to vary not only in different population groups but also geographically. Street children were investigated for *S. aureus* colonization and pathogens causing wound infections to establish baseline epidemiological information in Mwanza community.
Materials and Methods: Nasal and wound swabs were collected and processed to identify *S. aureus* carriage and pathogens causing wound infections respectively following standard operating procedures. A polymerase chain reaction was used to screen for Panton Valentine leucocidin (PVL) and *mecA* genes of 29 selected *S. aureus* isolates followed by spa typing.
Results: A total of 228 street children with the mean age of 14.09 ± 3.8 years were enrolled between April and July, 2015. Out of 228 street children, 109 (47.8%, 95% CI 38.2-56.9) had wounds of whom, 54(49.5%) had significant growth of pathogenic bacteria. *S. aureus* formed the majority 39/54 (72.2%) of the isolates. Other bacteria isolated were *Streptococcus pyogenes* (7), *Klebsiella* spp. (4), *Escherichia coli* (2) *Acinetobacter baumannii* (1) and *Achromobacter animicus* (1). Nineteen spa types were observed in 27 *S. aureus* from wounds that were typed. Spa type t690 (3) and t1346 (3) were the most frequent. Out of 39 *S. aureus*, 3(7.6%) were MRSA. All MRSA strains were typed as spa type t690. Two *S. aureus* strains were typed as new spa types t16294 and t16295. PVL was detected in 8/27(29.6%) of *S. aureus* isolates including in all MRSA isolates. Of 228 children, 27 (11.8%) were colonized with *S. aureus* of which only 2 had both colonization and infection.
Conclusion: There is a high diversity of *S. aureus* strains causing wound infections in street children residing in Mwanza city. MRSA spa type t690 which was previously detected in isolates from hospital might be the commonest cause of CA-MRSA wound infection within Mwanza region.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

802/MSP

Molecular epidemiology of mutations in antimicrobial resistance loci of *Pseudomonas aeruginosa* isolates from cystic fibrosis airways

L. Greipel[1], S. Fischer[1], J. Klockgether[1], M. Dorda[1], S. Mielke[1], L. Wiehlmann[1], N. Cramer[1], B. Tümmler*[1]
[1]*Medizinische Hochschule Hannover, Hannover, Germany*

The chronic airway infections with *Pseudomonas aeruginosa* in people with cystic fibrosis (CF) are treated with aerosolized antibiotics, oral fluoroquinolones and/or intravenous combination therapy with aminoglycosides and β-lactam antibiotics. An international strain collection of 361 *P. aeruginosa* isolates from 258 CF patients seen at 30 CF clinics was examined for mutations in 17 antimicrobial susceptibility and resistance loci that had been identified as hot spots of mutation by genome sequencing of serial isolates from a single CF clinic. Combinatorial amplicon sequencing of pooled PCR products identified 1,112 sequence variants that were not present in the genomes of representative strains of the 20 most common clones of the global *P. aeruginosa* population. A high frequency of singular coding variants was seen in *spuE, mexA, gyrA, rpoB, fusA1, mexZ, mexY, oprD, ampD, parR, parS* and *envZ (amgS)* reflecting the pressure upon *P. aeruginosa* in CF lungs to generate novel protein variants. The highest relative proportions of SNPs that are absent in the *P. aeruginosa* pangenome were found in *fusA1A2, mexA* and *pagL*. These genes encode proteins that are involved in unrelated activities, i.e. translation, transport and LPS modification, respectively suggesting that *P. aeruginosa* orchestrates a diverse armory to sustain in a hostile environment of host defense mechanisms and antimicrobial treatment. The CF lung habitat seems to favor diversifying selection in these targets of antimicrobial susceptibility and resistance. The mutation rate is elevated, about half of the singular SNPs are non-synonymous and the percentage of non-neutral amino acid exchanges is high. Our molecular epidemiology data point to an eminent role of private and *de novo* mutations of how the *P. aeruginosa* populations in the individual CF lungs try to escape the antimicrobial pressure.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

803/MSP

Genome-wide association studies for predicting complex antibiotic resistance from NGS data

R. Weber*[1], S. Fuchs[1], F. Layer[1], G. Werner[1], B. Strommenger[1]
[1]*Robert Koch Institute, Nosocomial Pathogens and Antibiotic Resistance, Wernigerode, Germany*

Introduction: Genome-wide association studies (GWAS) are a relatively new way for investigating the genetic background of resistance phenotypes in bacteria. Therefore, GWAS have been adapted for the application on bacterial populations over the last years. Some recently performed studies using GWAS were able to link elevated MICs for several antibiotics to the presence of particular single nucleotide polymorphisms (SNPs) which were subsequently used for resistance prediction. However, complex resistance mechanisms – like those for daptomycin – have been excluded from these studies so far.
Objectives: For *Staphylococcus aureus* the molecular mechanism of resistance towards daptomycin is not completely understood. Therefore, we initiated a GWAS using NGS data from a total of 99 *S. aureus* isolates to identify SNPs putatively associated with elevated daptomycin MICs.
Material and methods: Isolates were phenotypically characterized and selected according to their clonal background, isolation date and geographical region. Association testing was performed via PLINK and sequence element enrichment analysis. To account for population stratification in PLINK analysis, subpopulations within the overall population were identified with hierBAPS and covariates were used in association testing with the Cochran-Mantel-Haenszel test. Mesquite tool was used to identify homoplastic and rare SNPs within genes that are presumed to mediate daptomycin resistance.
Results: We were able to identify SNPs significantly associated with daptomycin-resistance. Those SNPs were predominantly located in the multipeptide resistance factor gene (*mprF*) that is well-known to be involved in the development of daptomycin resistance. As a proof of concept, we confirmed GWAS results by identifying homoplastic SNPs within *mprF*. However, for some isolates, we were not able to identify mutations that could be linked to daptomycin resistance. Therefore, we supposed the phenotype to be caused by other mutations that could not be detected by association at genome-wide significance levels. As a consequence, we searched for rare SNPs within genes expected to mediate daptomycin resistance. We could show that each isolate, showing a phenotype, carries at least one mutation within MprF protein. Interestingly, mutations within DNA-dependent RNA polymerase beta and the two-component signal transduction system YycFG could be identified in isolates showing cross-resistance towards vancomycin.
Conclusion: In this study we confirmed the potential of GWAS to identify resistance-associated SNPs. However, GWAS are not designed for the detection of rare SNPs. Therefore, additional approaches should simultaneously be applied to detect variants below genome-wide significance levels. Future work will focus on the characterization of substitution mutants in order to verify the influence of identified variants.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

804/MSP

Molecular epidemiology of vancomycin resistant enterococci in outbreak settings: Are SNP analyses an option?

S. Kampmeier*[1], A. Pettke[1], J. Schmitz[1], S. Bletz[1], A. Mellmann[1], A. Kossow[1]
[1]*Institute of Hygiene, University Hospital Münster, Münster, Germany*

Question: Vancomycin resistant enterococci (VRE) are an important cause of healthcare associated colonizations and infections. Whole genome sequencing (WGS)-based typing is used as an efficient tool to identify clonal relationships in non-outbreak settings. During VRE outbreaks, however, even using WGS-based typing the discriminatory power can find its limits due to the general low diversity of endemic VRE clones. Here, we investigate the discriminatory power of a single nucleotide polymorphism (SNP)-based analysis during a VRE cluster.
Methods: We identified a VRE cluster at the University Hospital Münster in September 2016. Twenty-two VRE isolates were subjected to WGS using the MiSeq platform (Illumina, USA). After sequencing, quality-trimming and *de novo* assembly, coding regions were compared in a gene-by-gene approach (core genome [cg]MLST) using the SeqSphere+ software version 3.0 (Ridom GmbH, Muenster, Germany). For SNP analysis, the online tool CSI Phylogeny 1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny/) was used to determine the SNP genotype. Strain AUS0085 (NC_021994) was used as reference sequence for SNP detection. Discriminatory index (*DI*) and display of the allelic differences in a minimum-spanning tree (MST) was conducted using SeqSphere+.
Results: WGS-based typing and MST analysis based on 1,670 of 2,547 genes, that were present in all isolates, resulted in 11 genotypes comprising two clusters with seven and 11 isolates, respectively, with a maximum of two alleles difference within the clusters. SNP genotyping resulted in 11 genotypes also comprising two clusters with seven and 11 isolates, respectively, with a maximum distance of six SNP within the clusters. *DI* of both methods were 0.861 and 0.866 for cgMLST and SNP typing,

respectively. In 19 of 22 isolates, both typing approaches were concordant.
Conclusions: VRE has epidemic potential, especially in high risk patients. Both WGS-based typing approaches reveal a similar discriminatory power supporting infection control strategies. Whereas cgMLST has a standardized nomenclature thereby enabling inter-laboratory comparisons, SNP-based approaches are only hard to standardize.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

805/MSP

Molecular biodiversity of drug sensitive and multridrug resistant *Mycobacterium tuberculosis* in Bulgaria: 2007-2016

S. Panaiotov*[1,2], E. Bachiyska[1], S. Yordanova[1], Y. Atanasova[1], Y. Todorova[1], V. Levterova[1], N. Brankova[1], I. Simeonovski[1], A. Karcheva[3], G. Refregier[2], C. Sola[2]
[1]*NCIPD, Microbiology, Sofia, Bulgaria*
[2]*Univ. Paris-Sud , Institute for Integrative Biology of the Cell (I2BC), Paris, France*
[3]*UMBAL, Pleven, Bulgaria*

Question: Long-term analysis of *M. tuberculosis* genetic biodiversity have not been performed in Bulgaria. The goal of our study was to investigate the phylogeographic spread of circulating genetic lineages of drug sensitive and multidrug resistant (MDR) *M. tuberculosis* strains across the country.
Methods: We analysed spoligo and MIRU-VNTR genotyping data of 597 drug sensitive and 283 multi-drug resistant *M. tuberculosis* strains collected during the past 10 years in Bulgaria. We assigned all isolates using TBminer and MIRU-VNTR*Plus* websites. We investigated major well documented historical immigrations to Bulgaria that occurred during the last ten centuries.
Results: Genetic profiles demonstrate that lineage 4 (Euro-American) is the only widely diffused in Bulgaria. Exceptions are 2 strains of *M. bovis* and 9 strains of the Beijing lineage 2 which was only detected recently (2007-2009). The prevalence of the Beijing genotype in Bulgaria is low. Not all detected cases of TB caused by the Beijing genotype were a result of human migration; MDR TB transmission within the country was also observed. We confirmed 50% prevalence of spoligotype SIT41, sublineage TUR among the MDR strains. Whole genome sequencing analysis confirmed a MDR outbreak associated with SIT41 genotype and that SIT41 is in circulation since 150 years.
Analysis of well documented immigrations of Roma from the Indian subcontinent in 10-12 century, Turks from Central Asia in the medieval centuries and more recently in the 20th century migrations of Armenians, Russians and Africans did not influence the biodiversity of *M. tuberculosis* in Bulgaria with genotypes of lineages 1, 2, 3, 5, 6 and 7.
Conclusions: SIT41 is a marker for MDR tuberculosis in Bulgaria. Several phylogeographically specific genotypes circulate in Bulgaria. Mass immigrations in Bulgaria during the last millennium did not affect the genetic biodiversity of *M. tuberculosis* with genotypes other than lineage 4. We hypothesize that immigrations in Bulgaria were not the main factor contributing to the genetic biodiversity of *M. tuberculosis*. More studies on factors controlling tuberculosis diversification including the local ecology are needed. The ecological factors leading to local genetic biodiversity in *M. tuberculosis* are multifactorial and not fully clarified. The reported data are valuable information for long-term management decisions for MDR tuberculosis control as a result of emergence and spread of endemic MDR genotype.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

806/MSP

A descriptive study on severe acute respiratory infections and influenza cases in Iran during seasonal flu epidemic of 2015-16

M. M. Gouya*[1], P. Hemmati[1], M. Nabavi[1], K. Seif Farahi[2], J. Ahmadi[3], R. Baiat[3], M. N. Dadras[1], F. Rezaei[1], M. Soroush[1], H. Akbari[1]
[1]*Ministry of Health & Medical Education, Iranian Center for Communicable Diseases Control, Tehran, Islamic Republic Of Iran*
[2]*Physician, Toronto, Canada*
[3]*Faranam Company, IT, Tehran, Islamic Republic Of Iran*

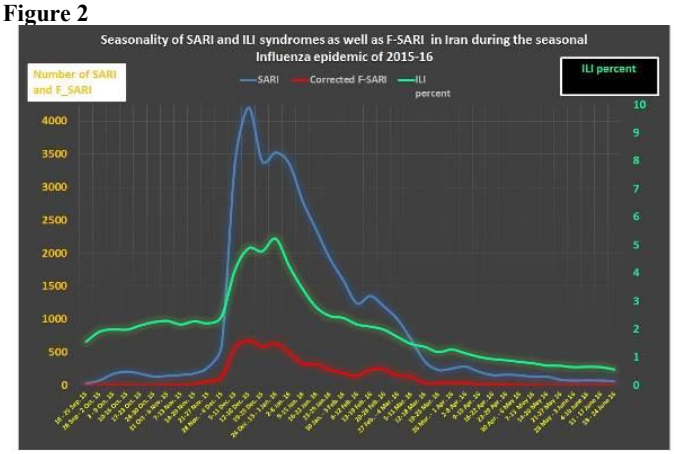
Question (Introduction & background): Globally, acute respiratory infections of lower respiratory system are the second cause of morbidity and third cause of mortality in all age groups. A significant proportion of global disease burden is attributable to influenza and RSV. In this study, we did a descriptive study on Severe Acute Respiratory Infection (SARI) and Flu-associated SARI (F-SARI) in I.R.Iran during the seasonal flu epidemic of 2015-16.
Methods: In order to gather SARI and F-SARI cases, we benefited the national influenza surveillance system. Countrywide, the cases were recorded by primary health care system of the country in hospitals during the seasonal flu epidemic starting early December 2015. Age and gender distribution in addition to background diseases and complications among hospitalized cases were described.
Results: Totally, 36692 SARI cases were recorded by national surveillance system of which 19578 were females versus 17101 males in the period of 20 September 2015 through 20 June 2016. Age distribution indicated that the most affected group were 25-34 (18.3%) and >65 (18.3%). In F-SARI cases the most common age group were 25-34 (21%), 35-44 (12.9%) and >65 (14.7%). Furthermore, some differences were observed between patients with some background diseases.
Discussion and conclusions: In previous year, the epidemic has mostly affected young and middle-age adults as well as elderly (beyond 65). Furthermore, those with chronic heart and respiratory diseases, pregnancy, diabetes and chronic renal diseases were the high-risk groups mostly affected. The epidemic trend demonstrated 3 waves of SARI and F-SARI among hospitalized cases. Circulated viral subtypes will be discussed during the conference.

Figure 1

Table 1. Number of Severe Acute Respiratory Illness (SARI) and Flu-Associated SARI (F-SARI) in the period from 20 September 2015 through 20 June 2016 in Iran

SARI	36692 cases (Male 17103 - Female 19589)
F-SARI (Positive for Influenza)	3665 cases (Male 1683 - Female 1982)
Sampled SARI cases (sampling fraction)	25267 (69% of SARI cases)
Estimated positive samples (F-SARI) in the epidemic season	5312 (14.5% of SARI cases)

Figure 2



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

807/MSP

Estimation of influenza and severe acute respiratory illness incidence (burden) in three provinces of the Islamic Republic of Iran, 2012 and 2013

P. Hemmati*[1], M. M. Gouya[1], A. A. Haghdoost[2], J. Ahmadi[3], K. Seif Farahi[4], R. Baiat[3], M. Nabavi[1], E. Mostafavi[5], T. Mokhtari Azad[6], F. Rezaei[1], H. Akbari[1], E. Bitaraf[7], H. Riazi[7], M. N. Dadras[1], M. Soroush[1], H. Barati[8], H. Shakoori[8], M. Rezvani[9], J. Bathaei[10]

[1]Ministry of Health & Medical Education, Iranian CDC, Tehran, Islamic Republic Of Iran
[2]Kerman University of Medical Sciences, Epidemiology, Kerman, Islamic Republic Of Iran
[3]Faranam Company, IT , Tehran, Islamic Republic Of Iran
[4]Physician, Toronto, Canada
[5]Pasteur Institute of Iran, Epidemiology, Tehran, Islamic Republic Of Iran
[6]Tehran University of Medical Sciences, National Influenza Center, Tehran, Islamic Republic Of Iran
[7]Ministry of Health & Medical Education, IT Department, Tehran, Islamic Republic Of Iran
[8]Karaj University of Medical Sciences, CDC, Karaj, Islamic Republic Of Iran
[9]Zanjan University of Medical Sciences, CDC, Zanjan, Islamic Republic Of Iran
[10]Hamedan University of Medical Sciences, CDC, Hamedan, Islamic Republic Of Iran

Question: A significant proportion of the global burden of acute lower respiratory infections is attributable to influenza and respiratory syncytial virus. There are few estimates of influenza burden in the World Health Organization Region for the Eastern Mediterranean. In this study we estimated the burden of severe acute respiratory infection (SARI) and flu-associated SARI (F-SARI) in selected provinces of Iran, the trends of SARI and confirmed cases of influenza (F-SARI) in 12 months (seasonality) and the age groups most at risk of SARI and F-SARI.
Methods: Using the electronic Iranian influenza surveillance system and data of cases in sentinel hospitals of 3 selected provinces, we estimated the monthly trend (seasonality) of incidence for SARI and F-SARI, overall incidence of SARI and F-SARI and their disaggregation by age with the aid using the Monte Carlo technique.
Results: The incidences for SARI and F-SARI for all age groups was 187.6 and 29.0 per 100 000 population, respectively.
Conclusions: A seasonal pattern in epidemics of influenza and SARI was observed similar to other countries of the northern hemisphere with several peaks in cold months. The age groups most at-risk were children aged under 2 years and adults older than 50 years.

Figure 1

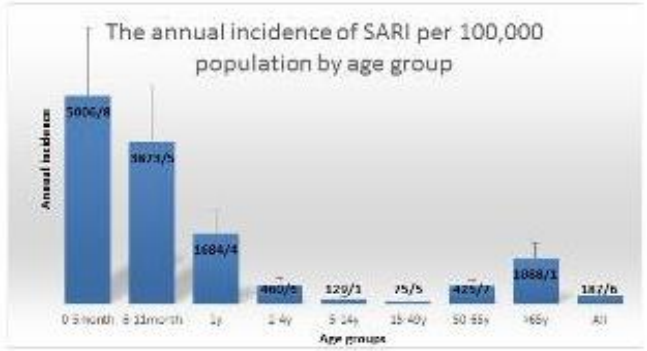
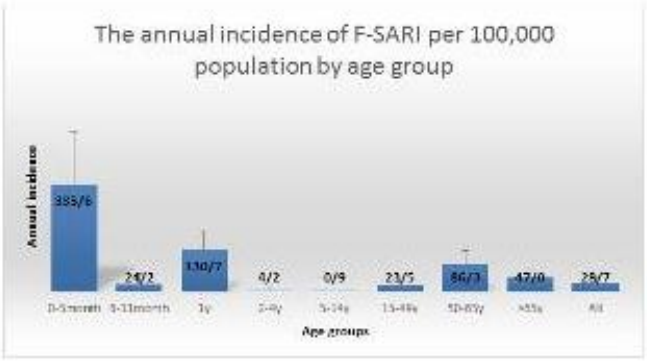


Figure 2



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

808/MSP

Influence of simulated microgravity on *B. subtilis* biofilms

F. M. Fuchs*[1], G. Holland[2], K. Madela[2], C. Falcón García[3], R. Hemmersbach[4], M. Laue[2], O. Lieleg[3], R. Moeller[1]

[1]German Aerospace Center (DLR e.V.), Institute of Aerospace Medicine, Radiation Biology, Köln, Germany
[2]Robert Koch Institute (RKI), Advanced Light and Electron Microscopy, Berlin, Germany
[3]Technische Universität München (TUM), Department of Mechanical Engineering and Munich School of Bioengineering, Garching, Germany
[4]Institute of Aerospace Medicine, German Aerospace Center (DLR e.V.), Gravitational Biology, Köln, Germany

Bacillus subtilis is one of the most studied Gram positive model organisms. Since mission Apollo 16, *B. subtilis* has been used for a multitude of space experiments. Investigating the influence of extreme conditions like those in space, non-domesticated strains, such as NCIB 3610 are of special interest regarding their ability to form biofilms. Since it is known that planktonic life is the exception, biofilms are considered as predominant way of living (Moons et al., 2009). Biofilms are organized in a complex self-produced extracellular polymeric matrix commonly composed of polysaccharides, proteins and nucleic acids. Building a biofilm protects the individual cell against shear forces, chemicals (e.g. antibiotics or disinfectants), temperature changes and water as well as nutrient depletion (Vlamakis et al., 2013, Cairns et al., 2014). The intrinsic resistance of biofilms is a problem, not only in industry and medicine, but it can be problematic under spaceflight conditions. Especially the loss of gravity coupled with changed levels of radiation might influence the resistance and therefore the virulence of bacterial biofilms. This can possibly evoke problems for the crew as well as for the spacecraft. In particular, long term missions with complex cooling systems, water supply and heat pipes may be vulnerable to biofilm colonisation.

In our work, we used the biofilm-forming wildtype strain NCIB 3610 and a biofilm-matrix deficient mutant (deletion of 15-gene exopolysaccharide operon, epsA-O) to study the impact of reduced gravity on maturated biofilms. Our major research goal is to compare biofilm formation in simulated microgravity (using a 2D clinostat) to terrestrial gravity (1g) conditions by using different microscopic techniques. White light profilometry, scanning and transmission electron microscopy (SEM, TEM) and confocal laser scanning microscopy (CLSM) were used to analyse biofilms regarding their topology and inner structure, respectively. First results show qualitative architectural differences between simulated microgravity and 1g in cross-sections, but no significant qualitative variations in biofilm surface topography.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

809/MSP

Development and evaluation of a novel vaccine against neoteric serotypes of *Streptococcus pneumoniae* prevalent in Egypt

R. Bahy[1], H. Hamouda[1], A. Shahat[2], A. Yassin*[3], M. Amin[3]
[1]*National Organization for Drug Control and Research, Microbiology, Giza, Egypt*
[2]*National Organization for Drug Control and Research, Biochemistry, Giza, Egypt*
[3]*Faculty of Pharmacy, Cairo University, Microbiology and Immunology, Cairo, Egypt*

Introduction: *Streptococcus pneumoniae* is still one of the major causes of morbidity and mortality worldwide especially among infants. The prevalent serotype distribution shows variation at different time intervals. In order to assess efficiently the epidemiology of the diseases for effective preventive and treatment strategies, serotype prevalence need to be periodically reassessed for the formulation of effective vaccines**.**
Objectives: The aim of this work is to determine the most recent serotypes of *Streptococcus pneumoniae* prevalent in Egypt, to prepare the conjugated capsular polysaccharide vaccine from these particularly predominant serotypes and evaluate them in vivo in an animal model.
Materials and Methods: Clinical specimens representing different cases of streptococcus infections were collected from the Greater Cairo area in Egypt. Conventional and molecular identification methods were performed, the antimicrobial susceptibility patterns were assessed and serotyping was done using PCR to identify the most prevalent serotypes. Capsular polysaccharides from the most current and prevalent serotypes were extracted, purified and conjugated to bovine serum albumin. The polysaccharide protein conjugates were purified through ultrafiltration technique and the molecular size distribution was determined compared to an available vaccine. The Immunogenicity of the prepared vaccine was examined in vivo by two different methods. First by measuring the elicited antibodies levels in blood after mice vaccination. Second by challenging the vaccinated mice groups with each serotype and determining the degree of protection offered by the developed vaccine.
Results: The results showed that among the clinical specimens collected, serotypes 6A/B and 19F were the most predominant. An alarming rise in antibiotics resistance among different isolates was observed. The conjugated capsular polysaccharide vaccine prepared from both serotypes revealed significant immunogenic effect in both in vivo methods examined. The vaccines prepared induced a rise in antibody levels as measured by Enzyme-linked immunosorbent assay (ELISA) and were able to increase the survival rate of the mice challenged with *Streptococcus pneumoniae* compared to appropriate animal control groups.
Conclusion: It is essential to track the most recent and prevalent serotypes of *Streptococcus pneumoniae* to prepare relevant, efficient and cost-effective vaccines particularly in developing countries.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Phage and CRISPR (FG PC)

810/PCP

A novel phage phiE72: an alternative therapeutic against *Staphylococcus epidermidis* infection and a potential research tool

X. Du*[1], V. Winstel[2], P. Kühner[1], Y. D. Stierhof[1], A. Peschel[1]
[1]*University of Tuebingen, Tuebingen, Germany*
[2]*University of Chicago, Chicago, Illinois, United States*

Staphylococcus epidermidis is one of the most common pathogens causing various types of nosocomial infections in hospitals, mainly by forming biofilms on medical devices. Nowadays, the situation of increasing number of *S.epidermidis* developed resistance to antibiotics is calling for alternative therapeutics. Besides, a novel research tool is also expected since study of the pathogenicity of *S.epidermidis* is limited due to genetic manipulation failure caused by strong genetic barrier mechanisms, especially the clinical ones. Recently, we isolated a new bacteriophage named phiE72 from a *S.epidermidis* strain in an infected tooth of a clinical patient. Electron microscopy revealed characteristics as bacteriophages of the *Siphoviridae* family. Phage infection assay using different bacterial species showed that phiE72 has a narrow host range and is specific to *S.epidermidis*. It showed a more drastical decrease of turbidity of bacterial host cell culture even compared to the widely studied antibiotic reagent member lytic polyvalent phage phiK. PhiE72 remained stable at pH values between 5.0 and 8.0 and up to the temperature of 60 °C . PhiE72 also showed tolerance to chloroform. The fast and strong lyse property, and specificity for *S.epidermidis* indicates the novel phage phiE72 an attractive candidate for phage therapy or as a biofilm eradication agent against *S.epidermidis*. Moreover, phiE72 can transduce plasmid DNA efficiently even to strains refractory to electroporation. Therefore, phiE72 might also become a valuable research tool for plasmid transduction for *S.epidermidis* strains, which are often difficult to transform.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

811/PCP

Isolation, characterisation and genomic analysis of bacteriophages against ESKAPE pathogens

J. Wittmann*[1], I. Schmidt[1], B. Bunk[1], C. Spröer[1], M. Rohde[2], C. Rohde[1]
[1]*Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany*
[2]*HZI - Helmholtz Centre for Infection Research, Central Facility for Microscopy, Braunschweig, Germany*

Introduction: The presence of multi-resistant bacteria, e.g. in the hospital environment, causing severe and life-threatening infections is a huge danger that urgently has to be overcome. The lack of new antibiotics against clinically relevant ESKAPE pathogens (*Enterobacter ssp.*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa, E. faecium*) calls for new medical approaches and agents to fight those. Bacteriophages are viruses that specifically infect and eliminate bacterial strains of one species and therefore might be an alternative to targetedly combat multi-resistant pathogens.
Objectives: This project aims to isolate and characterise bacteriophages against several multi-resistant ESKAPE pathogens, in particular *E. coli* and *S. aureus*, in order to evaluate

their potential to act as an alternative in the control of those pathogens. Besides lysis efficacy and host range analysis attention should particularly be paid to the genomic diversity of the isolated phages.
Material and Methods: Clinical strains were received from different hospital-associated collections and diagnostic laboratories (MH Hannover, Charité Berlin) and from the DZIF depository and open collection of the Leibniz-Institut DSMZ, Braunschweig. Phages were screened and enriched by inoculating bacterial cultures with 0.45µm- filtered environmental samples, e.g. sewage water, water samples and swabs from nasal cavities of different animals. Enrichment cultures were centrifuged and the filtered supernatant used for phage plaque assays. Further characterisation included host range analysis and electron microscopy. Several phages with promising host ranges were sequenced using the PacBio RSII system, their genomic data were analysed with different bioinformatic tools.
Results: In this study, we analysed several phages against *E.coli* isolated from various environmental samples that mainly belonged to the *Myoviridae* and *Siphoviridae* family, respectively, and revealed similarities to T4- and T5-like phages. Two phages, EBHT and MRLN, against MRSA strains, were isolated from nasal cavities of a pig and a horse, respectively. Both have a broad host range and lyse 62% and 95% of all tested *S. aureus* strains of this study. Genomic analysis confirmed their virulent character and classified them as a P68-like podovirus and a K-like myovirus.
Conclusion: We conclude from the phage lysis patterns there is therapeutic potential of some of the lytic phages with a rather broad host spectrum that we isolated from different sources. Especially the broad-host range phages EBHT and MRLN against MRSA are promising candidates for therapeutic approaches in the future. In regard to the cocktails against *E. coli* we conclude from the lysis phenotypes and genomic analysis that a diverse mixture of phages, via the diversity of host receptor types, might have potential to be efficient to biocontrol ESBL *E.coli* in poultry farming.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

812/PCP
An accessory wall teichoic acid glycosyltransferase protects Staphylococcus aureus from the lytic activity of Podoviridae
D. Gerlach*[1], X. Li[1], X. Du[1], J. Larsen[2], M. Stegger[2], P. Kühner[1], A. Peschel[1], G. Xia[1,3], V. Winstel[1]
[1]*Infection Biology, Tübingen, Germany*
[2]*Statens Serum Institut, Microbiology and Infection Control, Copenhagen, Denmark*
[3]*Institute of Inflammation & Repair, University of Manchester, Manchester, United Kingdom*

Many Staphylococcus aureus have lost a major genetic barrier against phage infection, termed clustered regularly interspaced palindromic repeats (CRISPR/cas). Hence, S. aureus strains frequently exchange genetic material via phage-mediated horizontal gene transfer events, but, in turn, are vulnerable in particular to lytic phages. Here, a novel strategy of S. aureus is described, which protects S. aureus against the lytic activity of Podoviridae, a unique family of staphylococcal lytic phages with short, non-contractile tails. Unlike most staphylococcal phages, Podoviridae require a precise wall teichoic acid (WTA) glycosylation pattern for infection. Notably, TarM-mediated WTA α-O-GlcNAcylation prevents infection of Podoviridae while TarS-mediated WTA β-O-GlcNAcylation is required for S. aureus susceptibility to podoviruses. Tracking the evolution of TarM revealed an ancient origin in other staphylococci and vertical inheritance during S. aureus evolution. However, certain phylogenetic branches have lost tarM during evolution, which rendered them podovirussusceptible. Accordingly, lack of tarM correlates with podovirus susceptibility and can be converted into a podovirus-resistant phenotype upon ectopic expression of tarM indicating that a "glycoswitch" of WTA O-GlcNAcylation can prevent the infection by certain staphylococcal phages. Since lytic staphylococcal phages are considered as anti-S. aureus agents, these data may help to establish valuable strategies for treatment of infections.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

813/PCP
Bacteria - Phage Interaction and the impact of Quorum Sensing
J. Feichtmayer*[1], L. Deng[1]
[1]*Helmholtz Zentrum München, Neuherberg, Germany*

The predatory pressure that phages impose on a bacterial population increases with increasing cell density. Likewise, fitness costs for carrying and expressing general phage resistance mechanisms are substantial and serve as a key factor in shaping the evolutionary dynamics between the phage and its host. Thus, bacteria have additionally evolved the ability to estimate the risk of a phage infection and adjust their strategies accordingly. An effective and widely-used bacterial communication and regulation system is the quorum sensing (QS), which is based on sensing the concentration of extracellular signaling molecules like e.g. N-acyl homoserine lactones (AHL). Prominent examples are the cell density controlled expression of antibiotics, bioluminescence or biofilm formation. QS-dependent downregulation of surface receptors important for phage infection could be shown for some bacteria (*Escherichia coli, Vibrio anguillarum*).
The aim of this project is to investigate the potential interplay between bacteriophages and the bacterial quorum sensing system. We postulate that bacteria use their QS system in the presence of a certain phage density in order to express anti-phage activities. Using an integrative approach of experimental and mathematical modelling tools, we investigated the underlying mechanisms of the interactions between phage infections and the QS.
We employed the model strain Pseudomonas putida IsoF and inoculated it with a mixture of five different bacteriophages, which were previously specifically isolated from untreated wastewater and were added after certain points in time: After 6h, when the QS was not yet induced; after 10h, when AHL concentration overstepped the threshold value and the QS system was activated; and after 14h, when AHL concentration decreases and QS is inactivated again. In another approach, bacteria were spiked with a certain amount of AHLs added to the inoculum before the critical cell density was reached (after 4h). Phages were then added after 5h. Bacterial growth was monitored via optical density measurements, viral concentrations were estimated using flow cytometry and AHL production was measured using an ELISA and a bacterial biosensor system over a time period of 34h.
We could observe a breakdown in AHL production right after the addition of the phage-mix. Although bacterial population densities recovered after a certain time, AHL production did not. The artificial addition of AHL alone and together with the phage-mix led both to a strong delay in the bacterial growth. Additionally, degradation of AHL molecules (activity of lactonases) seemed to be QS dependent as upon a certain AHL concentration and cell density, increasing concentrations of the degradation product were measured.
Incipient mathematical modelling indicated that phages clearly interfere with the AHL production to prevent the induction of some QS dependent (defense?) mechanisms. Especially, the reduction in the AHL concentration cannot be explained by merely the viral-induced cell lysis. On the other side, the addition of AHL prior to reaching a threshold cell density imposes

extensive metabolic costs without returning a gain upon viral infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

814/PCP

A novel strategy for exploitation of host RNase E activity by a marine cyanophage

C. Steglich*[1], D. Stazic[1,2], I. Pekarski[3], M. Kopf[1], D. Lindell[3]
[1]*University of Freiburg, Biology, Freiburg, Germany*
[2]*University of Stuttgart, Institut für Bioverfahrenstechnik, Stuttgart, Germany*
[3]*Technion, Biology, Haifa, Israel*

Previous studies have shown that infection of *Prochlorococcus* MED4 by the cyanophage P-SSP7 leads to increased transcript levels of host endoribonuclease (RNase) E. However, it has remained enigmatic if this is part of a host defence mechanism to degrade phage mRNA or if this single-strand (ss)RNA-specific RNase is utilized by the phage. Here we describe a hitherto unknown means through which this cyanophage increases expression of RNase E during phage infection and concomitantly protects its own RNA from degradation. We identified two functionally different RNase E mRNA variants, one of which is significantly induced during phage infection. This transcript lacks the 5"UTR, is considerably more stable than the other transcript, and is likely responsible for increased RNase E protein levels during infection. Furthermore, selective enrichment and *in vivo* analysis of double-stranded (ds)RNA during infection revealed that phage antisense (as)RNAs sequester complementary mRNAs to form dsRNAs, such that the phage protein-coding transcriptome is nearly completely covered by asRNAs. In contrast, the host protein-coding transcriptome is only partially covered by asRNAs. These data suggest that P-SSP7 orchestrates degradation of host RNA by increasing RNase E expression while masking its own transcriptome from RNase E degradation in dsRNA complexes. We propose that this combination of strategies contributes significantly to phage progeny production.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

815/PCP

Development of phage integration vectors for *Francisella tularensis*

H. Tlapák*[1], K. Rydzewski[1], K. Köppen[1], K. Heuner[1]
[1]*Robert Koch Institute, Berlin, Germany*

Introduction: *Francisella tularensis* (*Ft*) is the causative agent of tularemia. It can be found in a wide spectrum of wild animals and is able to infect humans through a variety of infectious routes, causing differing clinical expressions ranging from skin lesions to severe forms of pneumonia. In a recent study, we identified and described the genomic island (GI) FhaGI-1. We showed that its att sites in combination with the site-specific integrase are sufficient to generate the episomal form of the GI in *E. coli* and *Ft.* subsp. *holarctica* after transformation (Rydzewski, Tlapák et al., 2015; IJMM 305: 874-880).
Objectives: To utilize the knowledge about FhaGI-1 to generate phage integration vectors for use in *Francisella* research.
Material & Methods: *Francisella* strains were cultivated in medium T or on enriched cystine-heart agar supplemented with antibiotics, if necessary. Construction of the vectors was done by genetic cloning. Transformation of *E. coli* and *Francisella* strains was performed by electroporation.
Results: We constructed *Francisella* integration vectors using the essential parts of FhaGI-1. To enable propagation of the vector in *E. coli* we used vector pUC57-Kan as a basis. Furthermore, we inserted a multiple cloning site, as well as a second antibiotic resistance gene to allow for easy selection of clones, leading to the Francisella Integration Vector-tRNAVal-specific (pFIV1-Val). A sequence of positive and negative selection steps of transformants of *Ft. holarctica* LVS lead to 20-30 % of positive clones. Using a gfp+- construct we could demonstrate that the gene was still integrated and active after 10 passages in mediumT without antibiotic selection. Moreover, we could use the vector to successfully integrate *gfp* into other *Francisella* species (*Ft. novicida* Fx1 and U112, *F. philomiragia*). To further improve the vector, we cloned a *sacB*- gene into the non- integrating part of the vector to allow for easier selection of clones with only the integrated construct present but not the "empty" pUC57-Kan vector, leading to the second *Francisella* integration vector pFIV2-Val.
Conclusion: The results show that pFIV1-Val and pFIV2-Val can be used as a genetic tool in *Francisella* research, e.g. for stable complementation of specific gene- deletion mutants or to stably label bacteria.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

816/PCP

Regulatory interactions of *Corynebacterium glutamicum* and its prophages

M. Hünnefeld*[1], E. Pfeifer[1], J. Frunzke[1]
[1]*Forschungszentrum Jülich GmbH, IBG-1, Jülich, Germany*

Virus-derived DNA represents a predominant cause for strain-specific differences within a bacterial species. However, the integration of these DNA elements into the genome and into host regulatory circuits requires a stringent regulation.
The genome of the Gram-positive soil bacterium *Corynebacterium glutamicum* ATCC 13032 contains three prophages (CGP1-3). Among those, the large, cryptic prophage element CGP3 covers almost 6 % of the entire genome (~187 kbp) and is still inducible [1]. Prophage activation can be triggered both spontaneously and in an SOS-dependent manner [2]. Hitherto, current studies focus on the investigation of the molecular mechanisms underlying the control of prophage induction in *C. glutamicum* and its regulatory interaction with the host.
In recent studies we identified the small nucleoid-associated protein CgpS (CgpS: *C. glutamicum* prophage silencer), which was shown to act as an essential silencer of cryptic prophage elements in *C. glutamicum* [3]. ChAP-Seq experiments in combination with EMSA studies revealed that CgpS binds to AT-rich DNA and represses gene expression of mainly horizontally acquired genomic regions. Counteraction of CgpS activity by overexpression of the N-terminal oligomerization domain resulted in a severe growth defect and a highly increased frequency of CGP3 induction leading to cell death.
In recent attempts, we aimed at the identification of further transcriptional regulators interacting with CGP3. Interestingly, DNA affinity chromatography using promoter regions of various prophage genes revealed several host regulatory proteins binding to the CGP3 element. Among those, we identified prominent regulators of global stress responses and central carbon metabolism. These proteins illustrate the tight regulatory interaction of the host and its prophage. Current studies are aiming at a further functional analysis of selected candidates and their impact on CGP3 control.

References

[1] Helfrich, S., Pfeifer, E., Krämer, C., Sachs, C.C., Wiechert, W., Kohlheyer, D., Nöh, K., and Frunzke, J., *Live cell imaging of SOS and prophage dynamics in isogenic bacterial populations.* Mol Microbiol, 2015. 98(4): p. 636-50.
[2] Nanda, A.M., Thormann, K., and Frunzke, J., *Impact of spontaneous prophage induction on the fitness of bacterial*

populations and host-microbe interactions. J Bacteriol, 2015. 197(3): p. 410-9.
[3] Pfeifer, E., Hünnefeld, M., Popa, O., Polen, T., Kohlheyer, D., Baumgart, M., and Frunzke, J., *Silencing of cryptic prophages in Corynebacterium glutamicum.* Nucleic Acids Res, 2016.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

817/PCP
Assembly and activity of a minimal Type I-F CRISPR-Cas system
H. Müller-Esparza*[1], D. Gleditzsch [1], B. Turkowyd[1,2], U. Endesfelder[1,2], L. Randau[1]
[1]*Max-Planck-Institute for Terrestrial Microbiology, Marburg, Germany*
[2]*LOEWE Center for Synthetic Microbiology (SYNMIKRO), Marburg, Germany*

Introduction: Adaptive and heritable immune systems, termed CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR associated), are found in Archaea and Bacteria. Type I CRISPR-Cas systems mediate interference of invading genetic material by multi-protein complexes. These Cascade (CRISPR-associated complex for antiviral defense) assemblies contain different CRISPR RNAs (crRNAs) that screen foreign DNA (protospacers) via base complementarity. A sequence of two to five nucleotides (termed protospacer adjacent motif, PAM) is also recognised by the complexes and enables differentiation between self- and non-self DNA 1.
In most Type I CRISPR-Cas systems, PAM recognition is performed by a large Cascade subunit, which initiates R-loop formation between crRNA and its DNA target, leading to target degradation 2. Here, we provide mechanistic details for an unusual minimal Type I-F variant (I-Fv) CRISPR-Cas system from *Shewanella putrefaciens* CN-32. This systems Cascade comprises only 4 Cas proteins, but was shown to retain functionality against conjugative plasmids 3.
Methods: Heterologous Type I-Fv Cascade was produced in *Escherichia coli* and its activity against phage lambda and plasmids was tested. Crystal structures of the complex were obtained. The dynamics of fluorescently-labeled complexes were analyzed through SPT-PALM (single particle tracking photo-activated localization microscopy).
Results: PAM recognition and Cas3-dependent decay of phage lambda DNA was observed. In addition, the system was shown to impair plasmid transformation efficiency. Structural studies revealed unique Cas protein features that allow for efficient target recognition and Cascade backbone modulation. Finally, SPT-PALM analyses revealed different diffusion coefficients and distinct localization patterns for Cascade ribonucleoproteins in *E. coli.*
Conclusion: The co-evolution of viruses with their prokaryotic hosts led to the diversification of CRISPR-Cas systems. This study of a minimal Type I Cascade variant highlights novel targeting features that might balance protection from viruses with a reduced fitness cost.

References
[1] Horvath P., Barrangou R. CRISPR/Cas, the immune system of bacteria and archaea. Science 2010; 327:167-170.
[2] Hayes R.P., Xiao Y., Ding F., van Erp P.B.G, Rajashankar K., Bailey S., Wiedenheft B., Ke A. Structural basis for promiscuous PAM recognition in type I–E Cascade from E. coli . Nature 2016; 530:499-503.
[3] Gleditzsch D., Müller-Esparza H., Pausch P., Sharma, K., Dwarakanath S.,Urlaub H., Bange G., and Randau L. Modulating the Cascade architecture of a minimal Type I-F CRISPR-Cas system. Nucleic Acids Res. 2016; 44:5872-5882.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Microbiota, Probiota and Host (FG PW)

818/PWP
The probiotic E. coli strain Nissle 1917 inhibitis Shiga Toxin production in EHEC and protects E. coli K-12 Strains against stx-phage Infection.
S. Bury*[1], T. Oelschlaeger[1]
[1]*Instutute for Molecular Infection Biology, Infection Biology, Wuerzburg, Germany*

Enterohemorrhagic *E. coli* (EHEC), which are transmitted by contaminated food, have become a significant threat for humans as these pathogens can lead to the development of severe gastrointestinal disease and life threatening complications such as HUS. Since the large outbreak in Germany in 2011 a lot of research addressed the pathogenicity of EHEC and the development of new treatment strategies. The most important EHEC virulence factor is Shiga toxin (Stx), an AB5 exotoxin. Once secreted this toxin can bind with its B subunits to the globotriaosylceramide receptors (Gb3) of e.g. enterocytes and enter the cells by endocytosis. The A subunit has a specific N-glycosidase activity and cleaves an adenine base from the 28S rRNA of the ribosome by which the protein synthesis is blocked and the cells die due to apoptosis. Treatment of patients with antibiotics is not recommended as this is linked to an increase of released Stx [1]. Previous studies with probiotics showed *E. coli* Nissle 1917 (EcN) to inhibit both growth of EHEC strains and Stx production, which can only be traced back in part to the production of antibacterially operating microcins [2]. In the course of in vitro studies we could reveal that EHEC strains can convert *E. coli* K-12 strains to become Stx producers themselves which however, can be blocked by the presence of EcN but not by other commensal *E. coli* strains. This rescuing effect can be explained by an active reduction of *stx*-phages of EHEC provoked by the probiotic EcN, which could be demonstrated during culturing studies of EcN and isolated *stx*-phages. The in vitro results might reflect the in vivo situation where *stx*-phages can infect commensal bacteria in the human gut and turn them into Stx producers themselves. These findings encourage us to elucidate the mechanism of the downregulation of the Stx production in EHEC strains by EcN and support the idea of applying EcN as a medication in the treatment of EHEC infections as supplementary probiotic treatment during a human EHEC infection.

References
[1] DGI *EHEC und Antibiotikabehandlung*. 2011. 3.
[2] Rund, S.A., et al., *Antagonistic effects of probiotic Escherichia coli Nissle 1917 on EHEC strains of serotype O104:H4 and O157:H7.* International Journal of Medical Microbiology, 2013. 303(1): p. 1-8.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

819/PWP
Transcriptome analysis of *E. coli* Nissle 1917 in various conditions
M. Soundararajan*[1], T. Ölschläger[1]
[1]*Institut für Molekulare Infektionsbiologie, Würzburg, Germany*

E. coli Nissle 1917 (EcN) one of the best characterized probiotics and it is the active component of the probiotic preparation "Mutaflor®", which is used in the treatment of several gastrointestinal disorders. Studies have been reported on antagonistic activity of EcN against various Enterohaemorrhagic

E. coli strains (EHEC) such as the classical EDL933 and also isolates from the 2011 outbreak (Rund et al, IJMM, 2013) which emphasize EcN"s anti-pathogenic capability.
We believe, *E. coli* is very economical and effectors that affect host cells or pathogenic bacteria are produced only when necessary. The effectors ("tools") of EcN which are differentially regulated in the presence of pathogenic bacteria could be essential for its probiotic efficacy. So, the main objective of this study is to analyze the transcriptome of EcN under different mono and co-culturing conditions.
Initially, the transcriptome of EcN grown under fermenter condition was compared to the transcriptome of EcN grown at laboratory condition. And also, owing to the efficient antagonistic activity of EcN, we are interested in analyzing the changes in the transcriptome of EcN in co-culture with EHEC strain EDL933 and the nonpathogenic *E. coli* strain MG1655 in comparison with EcN mono culture. The co-culture experiments were performed in a transwell system and in order to narrow down the time point at which EcN starts exerting an effect on EDL933, a time point assay was performed. RNA was isolated from EcN incubated with just medium, or together with EDL933 and MG1655 using the QIAGEN RNAeasy mini kit and sent for sequencing and subsequent transcriptome analysis.
Analysis of changes in gene expression of the fermenter culture indicated that many gene clusters were differentially regulated when compared to LB-overnight culture. Specifically, there was a strong upregulation of the different iron uptake systems and curli fimbrial determinant which are necessary for effective colonization of the gut by EcN. In order to identify the time point at which EcN starts to influence the shigatoxin (Stx) level of EDL933, samples were taken from the co-culture for Stx determination by ELISA after 2 h, 4 h, 6 h, 8 h, 16 h and 24 h. And already after 5 hours of co-incubation with EcN a 73% reduction in Stx-level was observed. Hence the RNA was isolated from EcN under different co-culturing conditions after 3 h, 5 h, 7 h and 8 h of co-incubation. The importance of some of the genes of EcN which are differentially regulated in the presence of pathogenic bacteria will be evaluated by generating deletion mutants and corresponding complemented strains and testing these constructs in assays for Stx reduction.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

820/PWP
Investigation of sex-specific and sibling-related differences in the murine microbiota across body sites
A. Schöler*[1], A. Nayerova[1], M. Kostric[1], S. Krauss-Etschmann[2], M. Schloter[1]
[1]*Helmholtz Zentrum München, Environmental Genomics, Neuherberg, Germany*
[2]*Forschungszentrum Borstel, Borstel, Germany*

The human microbiome greatly contributes to our health by providing key metabolic functions, protecting us against pathogens and educating our immune system. Moreover, characterizing the human microbiome across different body sites revealed distinct microbial communities. Changes in the microbiome have been observed with many human conditions including obesity, infection and inflammation. It is therefore an active area of research to understand to what extent the human microbiome is influenced by lifestyle and genetic factors and several studies revealed important contributors such as gender or diet.
In this study we used mice as a model system to investigate gender-specific effects as well as sibling-related differences in microbiome structure. We analyzed four different body sites namely lung tissue, skin, mouth and caecum samples by 16s rRNA sequencing using the Illumina MiSeq platform. Our results confirm that microbiota composition depends on body habitat and differs between individuals. We further detect a more similar microbiome in siblings versus non-siblings and gender-related differences in the gut microbiota but no clear evidence for the other body sites.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

821/PWP
Purity makes the difference – Strategy of using LPS in *Galleria mellonella*
R. Parusel*[1], A. Schäfer[1], J. S. Frick[1]
[1]*Universitätsklinikum Tübingen, Institut für Medizinische Mikrobiologie und Hygiene, Tübingen, Germany*

The lepidopteran greater wax moth *Galleria mellonella (G.m.)* is a new and promising *in vivo* model organism in the field of host-microbiota interaction. *Galleria mellonella* larvae are suitable for several questions in innate immunity research including local or systemic infection. Low costs, easy handling and no conflicts with animal protection law or ethic guidelines raise the community of scientific users according to increasing numbers of publication. This organism is an auspicious candidate to reduce scientific used amounts of rodents in infection biology and contributes to the 3R strategy (Reduction, Refinement, Replacement) of animal experiments.
Lipopolysaccharides (LPS) of Gram negative bacteria are one of the most potent components for the activation of the host innate immune system and therefore LPS recognition is crucial for the host organism to clear infections of invading bacterial pathogens. LPS is also a common used stimulator of *Galleria mellonella* immune system and often used as prior stimulus in survival studies.
In our experiments we demonstrate high differences in using standard and ultrapure LPS of *Escherichia coli* in *Galleria mellonella*. To investigate whether varied compositions of LPS including ultrapure and standard LPS (contaminated with TLR2 ligands i.e. peptidoglycans according to manufactures description) influence the activation of innate immune system we stimulated *G.m.* larvae via hemolymph injection. The immune status after injection was analysed via several assays. For instance significant differences in mRNA expression levels of six antimicrobial peptides four hours after injection were detectable. Further an injection of high doses of standard LPS for more than 4 days leads to significant reduces survival rates while ultrapure does not. Therefore experiments with ultrapure LPS mixed with TLR2 ligands (synthetic and biological origin) to generate lethality were performed.
The induction of immune processes leading to death are probable not inducible with an ultrapure ligand. Thus these data suggest that induction of lethality is a multicomponent event. Furthermore the application of LPS as prior stimulus in standard quality and the effect on following survival studies should be scrutinized.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

822/PWP

Gastric bypass surgery markedly perturbs the community structure and the functional composition of the intestinal microbiota

S. Haange*[1,2], N. Jehmlich[1], C. Hintschich[3], M. Hankir[3], F. Seyfried[4], W. Fenske[3], M. von Bergen[1,2]
[1]*Helmholtz Centre for Enviromental Research, Department Molecular Systems Biology, Leipzig, Germany*
[2]*University of Leipzig, Institute of Biochemistry, Leipzig, Germany*
[3]*IFB Adiposity Diseases, Neuroendocrine regulation of energy homeostasis group, Leipzig, Germany*
[4]*University of Würzburg, Department of General, Visceral, Vascular and Pediatric Surgery, Würzburg, Germany*

Background and Question: Roux-en-Y gastric bypass (RYGB) surgery is used as a treatment for severe obese patients to help them lose excess weight. During this surgical procedure the anatomy of the stomach and upper intestine is rearranged which also leads to a change in the physical/chemical environment and nutrient sources present in the intestinal tract. This raises the question how these changes effect the community and functional structure of the intestinal microbiota?
Methods: To investigate these effects a RYGB rat model was used and the microbiota from the ileum, the cecum and the colon were sampled seperately, then investigated and compared to body weight matched animals with sham surgery. To analyse the community structure in regard to taxonomy and functionalities a multi-omics approach consisting of 16S rRNA gene sequencing, metaproteomics and metabolomics was used.
Results: The results reveal profound changes in the taxa distribution and the functions of the microbiota in all investigated gut sections after RYGB. In general, Actinobacteria were observed at higher, whereas Firmicutes were seen at lower abundances in RYGB. Though, *Clostridium perfringens* was far more abundant in RYGB. In the lower intestine we observed a higher concentration of amino acids as well as a change in the abundance of bacterial enzymes involved in amino acid metabolism and degradation. Furthermore, a number of bile acids were seen at significant lower concentrations in RYGB.
Conclusions: These results could be possibly helpful to minimize future bacterial infections, which are a concern after RYGB surgery.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

823/PWP

Differential cellular response (miRNAs, cytokines) of intestinal epithelial T84 and monocytic THP-1 cells to apical or basolateral infection by probiotic *E.coli* Nissle or enteropathogenic *E.coli* .

H. Sabharwal[1], M. A. Schmidt[1], C. Cichon*[1]
[1]*ZMBE, Institute of Infectiology, Muenster, Germany*

Introduction: The gastrointestinal tract is home to abundant and complex bacterial communities that contribute towards various immune and metabolic functions in the host. To confer protection against intruding pathogens, immunological host responses are activated by the pathogen-associated molecular pattern (PAMP) pathway via membrane-associated Toll-like receptors (TLR) resulting in the stimulation of nuclear factor kappa-B (NF-κB) signaling and the induction of a variety of pro-inflammatory cytokines like IL-8. The NF-κB pathway is central to the immune response and controls transcription of various cytokines and microRNA (miRNA) genes.
Objectives: Being at the sentinel location, the intestinal epithelial cells (IECs) mount an appropriate immune response to pathogenic microorganisms and at the same time avoid a damaging response to resident bacteria. How the gut mucosa is able to accomplish this complex task is poorly understood. The present study sheds light on the intricate cross-talk between the hosts immune system and exemplary pathogens and compares it to the host responses to probiotic bacteria.
Material & Methods: We characterized and compared exemplary cytokine and microRNA (miRNA) responses of human epithelial (T84) and monocytic cells (THP-1) toward the prototype enteropathogenic Escherichia coli (EPEC) strain E2348/69 and the probiotic strain Escherichia coli Nissle 1917 (EcN). Polarized T84 monolayers were infected apically or basolaterally.
Results: Bacterial challenges from the basolateral side resulted in more pronounced cytokine and miRNA responses than those observed for apical side infections. The probiotic EcN also caused a pronounced transcriptional increase of proinflammatory CXCL1 and interleukin-8 (IL-8) levels when T84 cells were infected from the basolateral side. MiR-146a, which is known to regulate adaptor molecules in Toll-like receptor (TLR)/NF-kB signaling, was found to be differentially regulated in THP-1 cells between probiotic and pathogenic bacteria. To assess the roles of flagella and flagellin, we employed several flagellin mutants of EcN. EcN flagellin mutants induced reduced IL-8 as well as CXCL1 responses in T84 cells, suggesting that flagellin is an inducer of this cytokine response. Following infection with an EPEC type 3 secretion system (T3SS) mutant, we observed increased IL-8 and CXCL1 transcription in T84 and THP-1 cells compared to that in wild-type EPEC.
Conclusion: Our study emphasizes the differential induction of cytokines and miRNAs by pathogenic and probiotic E. coli strains in epithelial and immune cells. Probiotics-induced miRNAs may pave the way for mediating mammalian host signaling and might foster new strategies for controlling host immune responses.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

824/PWP

Role of virulence factors, immune system and microbiota derived colonization resistance in *Yersinia enterocolitica* infection

J. Geißert*[1], S. Beier[2], E. Bohn[1], M. Schütz[1], D. Huson[2], I. B. Autenrieth[1]
[1]*University Clinics Tübingen, Interfaculty Institute for Microbiology and Infection Medicine, Tübingen, Germany*
[2]*University of Tübingen, Centre for Bioinformatics, Tübingen, Germany*

The dense and complex gastrointestinal (GI) microbial community is essential for development and maturation of a functional immune system. Beside the immune mediated indirect colonization resistance (CR), the microbiota may provide a direct CR against enteropathogenic bacteria like *Yersinia enterocolitica* (*Ye*). Ye virulence factors like the adhesin Yad A and the type-III-secretion system (T3SS) both contribute to effective colonization of the gut, invasion and abscess formation in lymphoid tissues after orogastral infection.
Aim of our project is to shed light on the role of these virulence factors for overcoming direct and indirect CR and for shaping the GI microbiota as well as host immune response. We use an orogastral co-infection model in mice with different colonization and immune states where 1:1 mixtures of *Ye* wildtype and mutant strains are injected and the colonization efficiency of gut and lymphoid tissues is followed up over 14 days. These colonization experiments indicate that Yad A and the T3SS are essential when facing an intact microbiota, but are dispensable under germfree conditions. In the absence of a fully developed innate immune response but in the presence of a microbiota the T3SS is crucial for colonization, in contrast to YadA. Gene expression analyses of inflammatory markers from intestinal mucosal scrapings are performed to quantify immune response in the different animal models. Based on the experimental data, we are developing a mathematical model for the predicition of bacterial population dynamics during *Ye* infection. To find out which constituents of

the commensal microbiota contribute to CR against *Ye*, we performed 16S rRNA and metagenome sequencing from small intestines of mice with either severe or mild outcome of *Ye* infection.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

825/PWP

Intestinal microbiota from broilers is shaped by substrate and end product of fermentation

K. Burbach*[1], D. Borda-Molina[1], E. Galster[1], S. Möhrmann[1], G. Matis[2], Z. Neogrady[2], K. Huber[1], A. Camarinha-Silva[1], J. Seifert[1]
[1]*University of Hohenheim, Institute of Animal Science, Stuttgart, Germany*
[2]*Faculty of Veterinary Science, Institute of Biochemistry and Physiology, Budapest, Hungary*

Question: The intestinal microbiome can influence the efficiency and health status of its host digestive system. By the host, indigestible non-starch polysaccharides (NSP) serve as substrates for bacterial fermentation resulting in short-chain fatty acids like butyrate. In broilers nutrition butyrate is of special interest for its beneficial impact on intestinal health and growth performance [1]. For those aspects, the utilization of dietary crude protein is as well relevant. In this study, 16S rRNA gene-based methods were used to evaluate the effect of broilers diets, varying in substrates for bacterial fermentation and butyrate supplementation, on the structure of the intestinal microbiota.
Methods: Eight groups, each with 10 broilers, were fed on either a maize-based or wheat-based diet, representing low and high NSP level, with adequate or reduced crude protein content and with or without sodium butyrate supplementation. Ileal and caecal digesta from 3 and 6 week old broilers were collected and genomic DNA was extracted according to previous findings [2]. For terminal restriction fragment length polymorphism (T-RFLP) analysis amplicons were digested by restriction enzyme HaeIII. Illumina amplicon sequencing was performed for the V1-2 region of the 16S rRNA gene [3]. Raw reads were quality filtered, assembled and analyzed using Mothur pipeline [4]. Data were analyzed on multivariate statistics based on Bray-Curtis resemblance.
Results: Comparison of the T-RFLP patterns and amplicon sequences revealed distinct differences by intestinal segment and variations within dietary groups. The overall structure of bacterial communities was statistically affected by diet (P= 0.001) and with both methods similarity analysis depicted a clear grouping by NSP source, but not by CP or butyrate. In caecal digesta, the Shannon diversity was lower in the wheat-based diets. *Bacteroides* increased its abundance in maize-based treatments while *Lactobacillus* and butyrate-producing members of the Lachnospiraceae were more abundant in wheat-based treatments. The presence of *Bifidobacterium* was characteristic for wheat-based treatments.
Conclusions: In this study the composition of diets affected the overall structure of broilers intestinal microbiota. The source of non-starch polysaccharides, and thereof the substrate for bacterial fermentation had a stronger stimulus on bacterial communities than CP content or supplementation with the fermentation product butyrate. Butyrate forming bacteria, belonging to Lachnospiraceae, showed to be stimulated with an enhanced amount of NSP in the wheat-based diets.

References

[1] Ahsan et al., World's Poultry Science Journal, 2016, 72:265-275.
[2] Burbach et al., MicrobiologyOpen 2016, 5:70-82.
[3] Camarinha-Silva et al., Environmental Microbiology, 2014, 16:2939-2952.
[4] Kozich et al., Appl Environ Microbiol, 2013. 79(17): p. 5112-20.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

826/PWP

Role of Intestinal Microbiota on Gut Barrier, Metabolic Function and Inflammation in a Humanized Mouse Model of Diet-Induced Obesity

V. Schüppel*[1], A. Hartstra[2], I. Lagkouvardos[3], M. Nieuwdorp[2], D. Haller[1,3]
[1]*TU Munich, Nutrition and Immunology, Freising, Germany*
[2]*University of Amsterdam, Internal Medicine, Amsterdam, Netherlands*
[3]*TU Munich, ZIEL-Institute for Food Health, Freising, Germany*

Introduction: Changes in gut microbiota composition and function have been linked to a variety of disorders, including obesity and diabetes. Colonization of gnotobiotic mice with obese human microbiota already provided some insights into functional aspects of host-microbe interactions in the context of obesity, but results are still inconsistent.
Objectives: The aim of this study was to establish a gnotobiotic mouse model for obesity and metabolic dysfunction using patient-derived stool microbiota before and after fecal transplantation.
Material & methods: Human stool samples were obtained from a fecal microbiota transplantation (FMT) trial at Amsterdam Medical Center including lean and insulin resistant patients with obesity (body mass index range of 30.3-41.7 kg/m2) and signs of systemic inflammation. Samples were collected before and after treatment using lean or autologous stools and microbiota composition was analyzed by 16S rRNA gene amplicon sequencing. Patients receiving either lean or autologous fecal microbiota plus butyrate tablets with improved insulin sensitivity and inflammation were selected. Patients remained obese independent of treatment. In order to generate a gnotobiotic mouse model for obesity and metabolic dysfunction germfree wild-type mice were colonized with patient microbiota for 8 weeks receiving control diet (CD). Mice remained on CD for additional 4 weeks or were switched to palm oil-based high fat diet (48 kJ%; HFD) in order to provoke diet-induced obesity.
Results: Mice on CD revealed normal body and fat pad weights associated with unaffected fasting blood glucose levels, an intact gut barrier and no signs of inflammation independently of the human donors. Additional challenge of colonized mice with HFD induced a significant increase in body and fat weight leading to impaired glucose tolerance, insulin resistance and elevated permeability of jejunum regardless of human microbiota source. Analysis of microbiota profiles showed an incomplete transfer of donor microbiota after transplantation of human microbiota into germfree mice characterized by a loss in number of bacterial species and changes in community structure, suggesting that obesogenic taxa remain within the group of non-transferrable bacteria including Clostridiales spp. (e. g. *Faecalibacterium prausnitzii*).
Conclusion: We demonstrated that obesity and insulin resistance cannot be initialized in mice by transferring patient-derived human fecal microbiota. In addition, the transfer of human microbiota into mice resulted in a substantial change of bacterial community structure and bacterial richness/diversity. Diet-induced impairment of glucose tolerance and gut barrier function was independent of patient donor microbiota.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

827/PWP

Therapeutic implementation of encapsulated *prtP*-encoded lactocepin on experimental colitis

H. Yoon*[1], J. Calasan[1], D. Haller[1], G. Hörmannsperger[1]
[1]*Technische Universität München, Chair of Nutrition and Immunology, Freising-Weihenstephan, Germany*

Introduction: *PrtP*-encoded lactocepin is a bacterial protease that has been shown to reduce experimental colitis via selective degradation of the proinflammatory chemokine IP10 upon oral therapy with lactocepin-expressing *Lactobacillus casei*.
Objective: The present study aimed to unravel the therapeutic potential of isolated *prtP*-encoded lactocepin in the context of inflammatory bowel diseases.
Methods: To enable the delivery of trypsin-sensitive *prtP*-encoded lactocepin to the large intestine, a pectin/zein-based encapsulation of lactocepin was developed. The impact of orally applied encapsulated lactocepin on colitis was then investigated in experimental models for acute colitis (DSS-induced) and chronic colitis (IL10-/- mice).
Results: Importantly, lactocepin was found to retain its anti-IP10 activity upon release from the pectin/zein capsules. *Ex vivo* incubation of pectin/zein capsules in cecum content of SPF mice demonstrated a continuous release of the capsule load within 24 hours. Experimental treatment studies revealed that daily oral application of encapsulated lactocepin (~50 µg) for seven days does not reduce the severity of DSS-induced colitis compared to placebo treatment. In IL10-/- mice, oral therapy with encapsulated lactocepin (~50 µg)(3x/week) for four weeks resulted in significant but moderate reduction of the histopathological colitis compared to analogous placebo treatment. TNF expression levels were found to be significantly reduced in large intestinal tissue of lactocepin treated IL10-/- mice, whereas systemic inflammation parameters (MLN weight, spleen weight, plasma serum amyloid A) were not affected by the treatment.
Conclusion: Oral therapy with encapsulated *prtP*-encoded lactocepin has a moderate anti-inflammatory impact on chronic colitis. We assume that the limited therapeutic efficacy is due to insufficient delivery of active lactocepin to the large intestinal mucosa. Oral application of genetically modified bacteria expressing high levels of active lactocepin at close proximity to the intestinal mucosa might therefore be a more effective treatment strategy.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

828/PWP

Characteristics of the dormant blood microbiota of healthy individuals

S. Panaiotov*[1], G. Filevski[2], M. Equestre[3], E. Nikolova[4], R. Kalfin[5]
[1]*National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria*
[2]*I Town Hospital, Sofia, Bulgaria*
[3]*Istituto Superiore di Sanita, Rome, Italy*
[4]*Bulgarian Academy of Sciences, Institute of Experimental Morphology, Pathology and Anthropology, Sofia, Bulgaria*
[5]*Bulgarian Academy of Sciences, Institute of Neurobiology, Sofia, Bulgaria*

Question: On the analogy of the non-pathogenic microbiota found in oral cavity, skin and gastrointestinal tract, existence of blood microbiota was confirmed by DNA sequencing, but never deeply characterized. Hypothesis for the existence of dormant blood microbiota in healthy humans have been arisen, but clear evidences of isolation on agar media have not been successful yet.
Methods: Several culture media were tested for isolation of blood microbiota**.** Resistance to physical and chemical treatments such as, beating at high speed with glass beats, heat, microwave and gamma irradiation, ultrasound disintegration, treatments with denaturing reagents, bases and acids were tested. Furthermore we evaluated five procedures for DNA isolation of the blood microbiota from cultures. Analysis for bacterial and eukaryotic species was performed by 16S rRNA, 18S rRNA and ITS2 sequencing.
Results: We developed successful isolation strategies of dormant blood microbiota**.** Blood microbiota were isolated in liquid cultures at 43 0C in 48 h and on agar plates prepared with homologous blood for 30 days at 43 0C. Media contained high concentrations of vitamin K. Isolation was equally effective from lysed or whole blood. Subculturing for the moment is not successful. On light microscopy the blood microbiota are observed after Gram staining. They parasitizes within erythrocytes, divide as free living and pass several morphological forms that represent different stages in their life cycle. The blood microbiota are resistant to denaturants, acids and bases, mechanical and ultrasound disintegration and can grow at unusual high temperatures. TEM images demonstrate well defined cell structures. On the bases of the sequencing results several taxonomy assignments for bacterial and eukaryotic species were given.
Conclusions: The dormant blood microbiota are innate of the healthy humans. Resident blood microbiota in healthy individuals should be considered non-pathogenic, of vital importance for the macroorganism and relatively constant over time. Its relative quantity or possible variation over time could play a role in the mutual relationship with the host and everyone may have a personal blood microbiota fingerprint. Interventional strategies to bind the host blood microbiome with the states of health and disease remain an unmet clinical need.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
National Reference Centres and Laboratories of Consultancy (StAG RK)

829/PWP

Strong antibacterial effect of non-antibiotic drugs on human gut commensals

L. Maier**[1], M. Pruteanu*[1], M. Kuhn*[1], A. Telzerow[1], K. C. Fernandez[1], G. Zeller[1], K. Patil[1], P. Bork[1], A. Typas[1]
[1]*EMBL Heidelberg, Genome Biology Unit & Structural and Computational Biology Units, Heidelberg, Germany*

The composition of the adult human gut microbiota remains fairly stable once established in early life, but can be altered as a consequence of disease, lifestyle changes or pharmaceutical uptake. Especially pharmaceuticals have recently emerged as one of the strongest contributing factors to microbiome composition, but data come mostly from single drugs, such as the antidiabetic drug metformin. In this study, we provide a comprehensive view of the effect of drugs on key species of the human gut microbiota by measuring the direct fitness effects of ~1200 marketed drugs on 40 representative strains (covering ~80% of the assignable average abundance of the human gut microbiome at genus level) in a one-by-one screening approach. Surprisingly, 24% of the non-antibiotic drugs affect growth of at least one species of our selection at low doses. Considerably more human-targeted drugs interfere with bacterial growth if doses are increased towards recommended administration levels. Interestingly, susceptibility towards antibiotics and non-antibiotic drugs correlates across bacterial species, suggesting common resistance mechanisms. Furthermore, we find that non-antibiotic drugs with antibacterial activity have a significant overlap in their side effect spectrum with antibiotics, and that in reverse, side effect patterns can be used to predict antibacterial activity of non-antibiotic drugs against our selected commensals. In conclusion, our findings offer

paths for mitigating microbiota-related side effects or for repurposing non-antibiotic drugs as antibacterials, and challenge our current view on emergence of antibiotic-drug resistance.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

830/RKP

***Corynebacterium* spp. nasal carriage in elderly people in Germany**

B. Teutsch[1], A. Berger[1], D. Marosevic[1], T. Lâm[2], S. Beer[3], N. Ackermann[1], S. Hörmansdorfer[1], H. Klaus[2], M. van der Linden[4], U. Vogel[2], A. Sing*[1]
[1]*LGL, Oberschleissheim, Germany*
[2]*University of Würzburg, Würzburg, Germany*
[3]*Bundeswehr, Koblenz, Germany*
[4]*University of Aachen, Aachen, Germany*

The prevalence of protective anti-diphtheria toxin antibodies decreases with age. Therefore, the elderly might serve as reservoir for potentially toxigenic *Corynebacterium* species. This study aimed to examine the colonization of the nasopharynx with corynebacteria of individuals older than 65.
In the period from October 2012 to June 2013, nasal and throat swabs were taken from 717 subjects aged 65-106 (average age 77.2) at 3 regions in Germany (Munich, Würzburg, Aachen) and investigated for *Corynebacterium* spp.. A questionnaire was used to gather information on individual factors which might have an impact on *Corynebacterium* carriage.
A total of 402 strains of *Corynebacterium* spp. were isolated from 387 subjects (carriage rate: 54%). The carriage rate was significantly higher in study participants living in retirement homes (67.7%) when compared to those living autonomously (50.9%). Moreover, in the age group > 80 years the carriage rate was significantly higher (61.3%) as compared to those aged 80 years and younger (50.5%). Strains were isolated mostly from the nose (99%). *C. accolens* was the most often isolated species (39.8%), followed by C. *propinquum* (24.1%), *C. pseudodiphteriticum* (19.4%) and *C. tuberculostearicum* (10.2%). No *C. diphtheriae, C. ulcerans* and *C. pseudotuberculosis* strains were isolated. A subsample of 74 subjects was tested serologically for anti-diphtheria antibodies. Protective anti-diphtheria toxin antibodies were found in 30% of the subjects. 70% of the subjects showed no protective immunity.
These results suggest that carriage of potentially toxigenic corynebacteria might be very rare among people aged 65 and older in Germany. However, the low prevalence of protective anti-diphtheria toxin antibodies might pose a risk for acquiring diphtheria especially for elderly persons.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

831/RKP

Report of the National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria on Carbapenemases in Germany in 2016

N. Pfennigwerth*[1], A. Anders[1], M. Korte-Berwanger[1], F. Lange[1], M. Kaase[1], S. G. Gatermann[1]
[1]*Ruhr-University Bochum, Department of Medical Microbiology, Bochum, Germany*

Question: Multidrug-resistance in *Enterobacteriacea*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since hardly any innovative antimicrobial drug against gramnegative 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 Laboratory for Multidrug-Resistant Gramnegative 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 β-lactamases.
Results: A total of 6535 isolates were investigated for carbapenemases in the National Reference Laboratory in 2016 until November 14th. Specimen sources were mostly rectal swabs (21.5%), urinary (16.1%) and respiratory samples (12.7%). Carbapenemases were found in 1386 *Enterobacteriaceae* strains (44.6%), 335 *P. aeruginosa* (24.0%) and 420 *A. baumannii* (94.1%). The most frequent carbapenemases in *Enterobacteriaceae* were OXA-48 (32.9%), VIM-1 (19.5%), KPC-2 (15.6%), NDM-1 (11.9%), OXA-181 (3.6%), KPC-3 (3.4%), NDM-5 (2.6%), OXA-162 (2%) OXA-232 (1.4%) and OXA-244 (1.3%). VIM-4, VIM-51, GIM-1, IMP-1, IMP-14, GES-5, OXA-58 and others were found in less than 1% each. In *P. aeruginosa,* VIM-2 was the most frequent carbapenemase (74.9%), followed by IMP-7 (4.8%) and GIM-1 (4.5%). NDM-1, GES-5, VIM-1, VIM-4, VIM-28, FIM-1, IMP-1, IMP-2, IMP-13 and IMP-22 were found in less than 2.1% each. OXA-23 was the most frequent carbapenemase in *A. baumannii* (79.5%), followed by OXA-72 (12.6%), OXA-58 (3.3%) and NDM-1 (3.1%). GES-11, GIM-1 and IMP-61 were found in less than 0.3% each.
Conclusions: A variety of different carbapenemases has established in Germany. However, the molecular epidemiology in Germany with a predominance of OXA-48 differs significantly from observations made in other countries like Greece, Italy or the USA. Compared to previous years, OXA-181 and OXA-232 are again on the rise, together with OXA-244.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

832/RKP

Establishment of the measurement of serum bactericidal antibodies after vaccination against serogroup B meningococci

H. Claus*[1], U. Vogel[1]
[1]*Institute for Hygiene and Microbiology, Würzburg, Germany*

Introduction: The multi-component meningococcal serogroup B vaccine (Bexsero) is available in Germany since December 2013. In 2015, the German Standing Committee on Vaccination (STIKO) recommended the use of Bexsero for persons with increased risk of meningococcal disease due to asplenia, complement deficiency, eculizumab therapy and laboratory contact. Vaccine antibody titres in these individuals are measured by serum bactericidal antibody (SBA) assays.
Objective: To establish an SBA assay for meningococcal serogroup B (MenB) at the reference laboratory for meningococci and Haemophilus influenzae (NRZMHi).
Materials & methods: Bactericidal antibodies against fHbp (factor H binding protein) were measured according to the standard protocol for meningococcal serogroups A, C, W, and Y except for the complement source, which is of human origin in the MenB assay. A reference serum from the National Institute for Biological Standards and Control (UK) together with titres ranges measured at the meningococcal reference unit in Manchester was used to determine accuracy and precision of the assay. Sera from vaccinated volunteers were used to study the performance of the assay.
Results: Among seven complement sources, only one fulfilled the criteria to be used in the MenB SBA assay. Sixteen assays conducted on 13 consecutive days with the reference serum with a designated titer of 1:32 to 1:128 gave the following titres: 1:32

(n=10), 1:64 (n=5) und 1:128 (n=1). Therefore, the accuracy and precision of the assay was evaluated as satisfactory. Of 30 vaccinees, 27 exhibited protective titres ranging from 4 to 256. Titres below 4 were observed in persons who had been vaccinated more than 530 before the measurement. This is consistent with the data of an antibody persistence study with Chilean adolescents, where the proportion of vaccinees with protective fHbp antibodies decreased to appr, 80% 18-24 months after vaccination with Bexsero (Santolaya et al. Hum Vaccin Immunother. 2013).
Conclusion: The B-SBA assay to measure protective antibodies induced by the meningococcal serogroup B vaccine Bexsero was successfully established at the NRZMHi.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

833/RKP
Investigation of *Vibrio cholerae* non-O1, non-O139 isolated from environmental sources and from food in Germany
K. Schwartz*[1], N. Bier[1], F. Schirmeister[1], A. Konietzny[1], E. Strauch[1]
[1]*Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany*

Question: Non-toxigenic *Vibrio cholerae* bacteria are present in German coastal waters and can be isolated from seafood. Most of the bacteria lack the cholera toxin gene (*ctx*) and the genes for the toxin-coregulated pilus (*tcp*). They are collectively designated non-O1, non-O139 strains. However, some strains can cause gastroenteritis and extraintestinal infections. In the present study, we investigated German isolates to find out if some of these isolates could pose a risk for public health.
Methods: We selected 100 environmental strains from the North Sea and Baltic Sea and 30 isolates from seafood. The strains were characterized by MLST and examined for the presence of cholera toxin gene and other virulence-associated factors including hemolysins, RTX toxins, pandemic islands and type III secretion system. Phenotypic assays for hemolytic activity were also performed.
Results: Genotyping results showed that none of the isolates contained the cholera toxin (*ctxA*) and genes of the *ctx* associated element as well as those for the toxin-coregulated pilus. The presence of other toxins showed a strain specific pattern. Based on MLST analyses, the phylogenetic relationship of strains was characterized. Nearly all strains showed clear hemolytic activity against human and sheep erythrocytes.
Conclusion: Our study indicates the need for continued surveillance of *Vibrio* spp. in Germany as *Vibrio* infections are predicted to increase due to global warming.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

834/RKP
Serogroup W meningococcal disease in Germany
H. Claus*[1], T. Lâm[1], U. Vogel[1]
[1]*Institute for Hygiene and Microbiology, Würzburg, Germany*

Introduction: Serogroup W Meningococcal (MenW) disease is rare in Germany with only 3.5% of all cases in 2012-2015. England and Wales experienced an increase of MenW disease with sequence type (ST)-11 strains (the South American-UK clone) from 1.8% of all cases 2008/2009 to 15% 2013/14 (Ladhani et al. Clinical Infectious Diseases 2015). The UK therefore initiated an adolescent vaccination program (Ladhani et al. Arch Dis Child 2016).
Objectives: Analysis of the dataset of the reference laboratory for potential recent increases of MenW disease in Germany (2015 and 2016) as experienced in England and other European countries
Materials & methods: MenW isolates submitted to the German reference laboratory for meningococci and Haemophilus influenzae (NRZMHi) were analysed by finetyping (serogroup:PorA-variable region (VR)1 and VR2: FetA-VR) and multi-locus sequence typing (MLST).
Results: The number of MenW cases in Germany increased from 10 (3.7 % of all cases) in 2015 to 22 (10.5%) in 2016 (as of October 2016). In both years, W:P1.5,2:F1-1 was the dominant finetype with 4 of 10 cases (40%) in 2015 and 9 of 22 cases (41%) in 2016. In 2015, 5 of 8 and in 2016, 11 of 22 isolates that could be analysed by MLST belonged to the ST-11 complex. There was no evident spatial clustering of cases. Median age was 33 yr (25 percentile: 17 yr; 75 percentile: 66 yr).
Conclusion: MenW disease continues to be rare in Germany. Nevertheless, there was an increase in 2016, which needs to be followed carefully. However, the ST-11 complex is only partially responsible for this increase. Genome sequencing will be applied to map the circulating strains to the South American-UK clone. The age distribution of MenW cases was atypical for meningococci; there was no difference in the age distribution between ST-11 complex cases and other complexes.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Regulation and Signal Transduction in Prokaryotes (FG RS)

835/RSP
Conversion of adrenaline by *Vibrio cholerae* to adrenochrome, a novel effector molecule
J. Steuber*[1], C. Toulouse[1]
[1]*University of Hohenheim, Institute of Microbiology, Stuttgart, Germany*

Question: Host-derived stress hormones such as adrenaline (A) and noradrenaline (NA) stimulate growth and swarming of enterobacteria like *EHEC* (1) or *Salmonella* enterica Typhimurium (2), and of *Vibrio cholerae* (3), the causative agent of the Cholerae disease. Adrenochrome (AC), an oxidation product of A, was identified in *V. cholerae* supernatants (3). Here we investigated the mechanism of AC formation, and its effect on *V. cholerae* growth, motility and toxin production.
Methods: *V. cholerae* was grown aerobically in M9 minimal medium with glucose as carbon source with 20 or 100 µM adrenochrome. Motility of *V. cholerae* in the presence of A, NA or AC was followed on soft agar plates containing bovine serum. The B-subunit of cholera toxin was quantified in *V. cholerae* supernatants by ELISA. NADH oxidation and AC formation by the Na^+ -translocating NADH:quinone oxidoreductase (NQR) was followed spectrophotometrically.
Results: AC was not utilized as carbon source by *V. cholerae*, but increased growth rates on glucose up to two-fold. We observed significant ($p < 0.05$) increase of diameters of swarming rings of *V. cholerae* with NA and AC, but not with A. Cholera toxin production was slightly increased with AC (0.29 ± 0.09 µg/ml) compared to A (0.24 ± 0.01 µg/ml), NA (0.20 ± 0.01 µg/ml) or the control without addition (0.22 ± 0.02 µg/ml). The respiratory NQR of *V. cholerae* catalyzed NADH-dependent AC formation in the presence of O_2 and A. Anoxic conditions, or addition of superoxide dismutase, prevented AC formation by NQR, indicating that superoxide produced by NQR during NADH oxidation converted A to AC in a non-enzymatic reaction.
Conclusion: Increased susceptibility of a stressed host to bacterial infections is well-documented. Underlying mechanism(s) are likely to include inter-kingdom signaling between the host and the pathogen by stress hormones. We demonstrate that the stress hormone adrenaline is readily converted by *V. cholerae* to adrenochrome in a respiration-triggered, superoxide-dependent reaction. AC stimulates growth and motility of *V. cholerae* and therefore is expected to facilitate colonization of the human host.

We speculate that the respiratory burst by immune cells counteracting the infection further increases AC formation *in vivo*.

References
[1] Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V. 2006. The QseC sensor kinase: a bacterial adrenergic receptor. PNAS 103:10420-10425.
[2] Moreira CG, Weinshenker D, Sperandio V. 2010. QseC mediates *Salmonella enterica* serovar typhimurium virulence *in vitro* and *in vivo*. Inf. Imm. 78:914-926.
[3] Halang P, Toulouse C, Geissel B, Michel B, Flauger B, Muller M, Voegele RT, Stefanski V, Steuber J. 2015. Response of *Vibrio cholerae* towards the catecholamine hormones epinephrine and norepinephrine. J. Bacteriol. 197:3769-3778

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

836/RSP
Functional important interaction sites of the nitrate sensor NreA of *Staphylococcus carnosus*
C. Barth*[1], G. Unden[1]
[1]*Johannes Gutenberg-Universität Mainz, Institut für Mikrobiologie und Weinforschung, Mainz, Germany*

The anaerobic nitrate respiration of *Staphylococcus carnosus* is regulated by the two component system NreBC in response to O2 and the nitrate sensor NreA. NreB and NreA form a sensor complex and the activity of the sensor kinase NreB is influenced by the interaction with NreA in a nitrate dependent manner. Binding of nitrate to NreA leads to an altered interaction of the NreA homodimer and of the NreA/NreB heterodimer [1, 2]. NreA has two highly conserved surface and potential interaction sites. NreA variants of the supposed interaction sites were produced by site directed mutagenesis and tested *in vivo* using BACTH interaction assay. The results indicate that the cluster around the nitrate binding pocket of NreA is important for the interaction between NreA monomers. The second cluster is important for NreA/NreB heterodimerization.

References
[1] Nilkens, S., Koch-Singenstreu, M., Niemann, V., Götz, F., Stehle, T. & Unden, G., (2014) *Mol Microbiol* 91:381-393
[2] Niemann, V., Koch-Singenstreu, M., Nilkens, S., Götz, F., Unden, G. & Stehle, T. (2014) *J Mol Biol* 426:1539-1553

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Secondary Metabolites (FG SM)

837/SMP
Genomic and metabolomic analysis of *Termitomyces* sp., the fungal mutualist of *Macrotermitinae*
N. Kreuzenbeck*[1], V. Challinor[2], H. Hu[2], S. Otani[2], M. Poulsen[2], C. Beemelmanns[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology-HKI, Jena, Germany*
[2]*University of Copenhagen, Biology, Copenhagen, Denmark*

Introduction: Fungus growing termites (*Macrotermitinae*) cultivate a symbiotic fungus (*Termitomyces* sp.) for nutrition. The fungus is grown on predigested plant material, which is piled up by the termites as a comb-like structure to enable optimal growth conditions.[1] This environment is prone to exploitation by parasitic fungi (e.g. *Pseudoxylaria* sp.), if the colony ecosystem is out of balance.[2] Due to the longevity and stability of a healthy termite colony, we hypothesize that the symbiotic food fungus *Termitomyces* sp. contributes to garden defense beside the termites and other microbial commensals or symbionts, by the production of e.g. antimicrobial natural products.[3]
Objectives: We are investigating the biocatalytic potential of *Termitomyces* sp. on a genomic, transcriptomic and metabolomic level to understand the ecological role of the encoded natural products in the ancient mutualistic relationship.
Materials and methods: Based on available genome information, we analyzed the secondary metabolite repertoire. We then performed transcriptomic studies coupled with LC-MS and NMR based metabolomics studies.
Results: Genome mining revealed the presence of PKS, NRPS, NRPS-like and terpene synthase biosynthetic genes. The expression of genes located in PKS and NRPS gene cluster was confirmed and the expression of NRPS-like and terpene synthase genes is now under investigation. In parallel the metabolome capacity of *Termitomyces* sp. was investigated by a comparative cultivation approach that revealed the time- and media-dependent production of a great number of different metabolites.
Conclusion: We were able to establish a reliable method for studying the biosynthetic repertoire of *Termitomyces* sp., which will be applied to identify the encoded natural products.

References
[1] a) Aanen, D. K.; Eggleton, P.; Rouland-Lefèvre, C.; Guldberg-Frøslev, T.; Rosendahl, S.; Boomsma, J. J. *Proc. Natl. Acad. Sci. U. S. A*. 2002, *99*, 14887–14892; b) Poulsen, M.; Hu, H.; Li, C.; Chen, Z.; Xu, L.; Otani, S.; Nygaard, S.; Nobre, T.; Klaubauf, S.; Schindler, P. M.; Hauser, F.; Pan, H.; Yang, Z.; Sonnenberg, A. S. M.; de Beer, Z. W.; Zhang, Y.; Wingfield, M. J.; Grimmelikhuijzen, C. J. P.; de Vriese, R. P.; Korb, J.; Aanen, D. K.; Wang, J.; Boomsma, J. J.; Zhang, G. *Proc. Natl. Acad. Sci. U. S. A*. 2014, *111*, 14500–14505.
[2] Visser, A. A.; Kooij, P. W.; Debets, A. J. M.; Kuyper, T. W.; Aanen, D. K. *Fungal Ecol* 2011, 4(5), 322-332.
[3] a) Beemelmanns, C.; Guo, H.; Rischer, M.; Poulsen, M. *Beilstein J Org Chem* 2016, 12, 314-327; b) Ramadhar, T. R.; Beemelmanns, C.; Currie, C. R.; Clardy, J. *J Antibiot (Tokyo) 2014,* 67(1), 53-58.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

838/SMP
Studies on the composition of ClpP complexes and their ADEP sensitivity in *Streptomyces hawaiiensis* NRRL 15010
L. Reinhardt[1], D. Thomy*[1], P. Sass[1], H. Brötz-Oesterhelt[1]
[1]*University of Tuebingen, Department for Microbial Bioactive Compounds, Tuebingen, Germany*

The acyldepsipeptide antibiotic ADEP1 was discovered as main component of the antibacterial complex A54556 produced by *Streptomyces hawaiiensis* NRRL 15010 [1]. ADEPs have potent antibacterial activity against Gram-positive bacteria, including multi-drug resistant clinical isolates [2].
ADEPs target the proteolytic core of the bacterial caseinolytic protease (ClpP) via a dual mechanism. By using the same binding sites as the associated Clp-ATPases ADEPs inhibit all natural functions of ClpP. At the same time, they induce conformational changes in the ClpP core leading to an opening of the entrance pore to the proteolytic chamber of the ClpP tetradecamer and allosteric activation of the catalytic triade [3]. Depending on the species, cells die either due to uncontrolled ClpP activation or due to the loss of indispensable natural functions of ClpP [4].
In this study, we investigate the ClpP complex composition of the ADEP producer strain *S. hawaiiensis*. *Streptomyces lividans,* which depends on a functional ClpP for viability, was shown to encode 5 different ClpP homologs, organized in two bicistronic and one monocistronic operon [5]. Here, we analyzed the *clpP*

genes of *S. hawaiiensis* and observed high sequence homologies to *S. lividans* as well as the same gene organization.
In *S. lividans* the expression of ClpP1/2 and ClpP3/4 is regulated by distinct transcription factors and expression of either ClpP1/2 or ClpP3/4 is essential for viability [5]. This raises the question of interaction and functional cross talk between the ClpP proteins in *Streptomyces*. So far, none of the *Streptomyces* ClpP proteins was studied *in vitro*. Here, we expressed native and tagged versions of ClpP1 and ClpP2 recombinantly in *E. coli* and purified them via anionic exchange chromatography or their C-terminal histidin-tag. Studies on the oligomeric state via gelfiltration showed that both ClpP1 and ClpP2 assemble as homo-tetradecamers. In further studies we want to determine the composition of the proteolytically active complex(es) *in vitro* and test their sensitivity against ADEP.
Therefor we established a fast purification procedure for ADEP1 from *S. hawaiiensis* culture broth as a prerequisite in peptide and protease activity assays as well as in antimicrobial activity tests.

References
[1] Michel et al (1985) US Patent # 4492650
[2] Brötz-Oesterhelt et al (2005) *Nat Medicine,* 11 (10): 1082-1087
[3] Gersch et al (2015) *Nat Commun*, 6: 6320
[4] Famulla et al (2016) *Mol Microbiol*, 101(2):194-209
[5] Gominet et al (2011) *Microbiology*, 157: 2226-2234

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

839/SMP
Development of reporter strains for the identification of novel antibacterial agents.
K. Wex*[1], A. Berscheid[1], H. Brötz-Oesterhelt[1]
[1]*Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), Microbial Bioactive Compounds, Tübingen, Germany*

The Tuebinger strain collection includes a large number of proven producers of interesting compounds, which have not been followed up. So far, these strains were only grown in standard production media and no effort has been made to analyze their full biosynthetic capabilities. We are developing methods to exploit this potential. A special effort will be made to develop a robust reporter system for antibiotic production.
Ideally, this system should allow us to detect the production of antibiotics, while the fermentation process is still ongoing. Promoters are known from previous large-scale transcriptome studies, that are selectively induced upon stresses acting on the four main bacterial metabolic pathways: RNA synthesis, DNA synthesis, protein synthesis or cell wall synthesis. So far, such reporter strains have been employed predominately for mode of action studies of antibacterial agents, providing first ideas on the metabolic pathway affected (1,2). We now are exploiting reporter strains to monitor production of antibiotic substances to screen for isolates that produce antibacterial agents and to select conditions favorable for antibiotic production. In a feasibility study we are constructing a panel of reporter strains to be used in the screening effort of the Tuebinger strain collection, covering not only the four main pathways, but also inhibitors of the fatty acid synthesis.

References
[1] Urban, A. *et al.* Novel Whole-Cell Antibiotic Biosensors for Compound Discovery. *Applied and Environmental Microbiology* 73, 6436-6443, doi:10.1128/AEM.00586-07 (2007).
[2] Hutter, B. *et al.* Prediction of Mechanisms of Action of Antibacterial Compounds by Gene Expression Profiling. *Antimicrobial agents and chemotherapy* 48, 2838-2844, doi:10.1128/AAC.48.8.2838-2844.2004 (2004).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

840/SMP
Characterization of the *Pseudomonas aeruginosa* c-di-GMP binding protein FlgZ that interferes with bacterial motility
S. Bense*[1], J. Düvel[1], S. Häußler[1]
[1]*Helmholtz Centre for Infection Research, Molecular Bacteriology, Braunschweig, Germany*

Question: The second messenger c-di-GMP is an important bacterial signaling molecule that is involved in the regulation of motility versus sessility. High intracellular levels of c-di-GMP are associated with a sessile lifestyle within biofilms while low levels of c-di-GMP promote motility and virulence. The c-di-GMP signal is translated into a cellular response by c-di-GMP effectors. How these effectors mediate downstream effects upon c-di-GMP binding often still has to be defined. In this study, we focus on the c-di-GMP binding protein FlgZ that belongs to a prominent class of effector proteins, the PilZ domain proteins. We characterize this protein on the level of transcriptional regulation as well as on the functional level.
Methods: For the investigation of the transcriptional regulation we analysed transcriptional profiles and the operon structure, determined the transcriptional start sites (TSS) and measured promotor activity in different sigma factor knockout strains.
For the phenotypic characterization we applied motility assays on different strain backgrounds. With a prolonged motility assay we generated a motile suppressor mutant. Whole genome sequencing was used to map the underlying mutation to a coding sequence of the PA01 genome.
Results: Global gene expression analyses indicated that *flgZ* is the third gene of the *flgMNZ* operon and that transcription initiation of the *flgMNZ* genes is driven by the alternative sigma factors RpoF and RpoN. However, we identified an alternative TSS that is located within the operon structure and downstream of the annotated start codon of FlgZ. Confirmation of this alternative TSS by 5´RACE and analysis of the protein length by the use of polyclonal antibodies lead to the re-annotation of the translational start site of the FlgZ protein. Furthermore, we found that changing environmental conditions can change transcription initiation of *flgZ* from the promoter of the *flgMNZ* operon to the promoter directly upstream of *flgZ*, whose transcription is then decoupled from the *flgMN* genes.
In a Δ*flgZ* mutant we observe an improved swimming and swarming motility while overexpression of *flgZ* shows a negative influence on these flagellar mediated types of motility. The generated motile suppressor mutant did not show the inhibitory effect of a *flgZ* overexpression. This mutant harbours a mutation in a gene which gene product plays a role in twitching motility and functions as a polar anchor.
Conclusions: The analysis of the transcriptional regulation reveals a flexible system for the expression of the *flgMNZ* operon. Additionally, we could demonstrate that FlgZ needs to be re-annotated. On the basis of the gene product of the suppressor mutant we gain insights regarding motility regulation.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

841/SMP

Cryo preservation techniques for secondary metabolite producing eukaryotes

S. Schmidt[1], M. Schramm[1], I. Mannott[1], A. Labes*[1]
[1]*Hochschule Flensburg, FB2 Energie und Biotechnologie, Flensburg, Germany*

Question: Natural products are the starting points for drug development. They can also be applied in cosmetics, help to replace toxic chemicals or can be applied as plant protecting agents and many other application fields. In recent years, the great need for new natural products, mostly being derived from secondary metabolism, led to a revival of natural product research including an increased isolation of new producer strains. Both, bacteria and eukaryotic microbes contributed to this pool of new compounds. One successful strategy is to search for new biodiversity in understudied or extremophilic habitats. Just statistically, extreme or non-accessible environments are scarcely studied, increasing the probability of finding new taxa and thereby also new metabolites and enzymes. Preservation of newly isolated strains is crucial for avoiding genetic changes during repeated cultivation and as a sustainable source for all subsequent steps including biotechnological upscaling.
Unlike most other groups of microorganisms, eukaryotic microbes, especially microalgae and fungi have traditionally been maintained by routine serial subculture (1). Even more, many of them show quite low viability, when using classing cryopreservation techniques.
Methods/Results: With the focus on secondary metabolite producing fungi and microalgae, we review and study the probability of a variety of cryotechniques in order to define standard starting points for cryopreservation strategies. Addition of stabilisers, freezing stragegies and surface provision methods were applied, revealing that the cryo preservation techniques are applicable but must be adapted to each taxonomic group.
Conclusions: Specific preservation techniques must be applied reflecting on the cellular biology of the strain to be preserved. More efforts should be undertaken to develop specific techniques for specific groups.

References

[1] J. Day, Cryopreservation of Microalgae and Cyanobacteria, in: Cryopreservation and freeze-drying protocols / edited by John G.Day, Glyn N. Stacey. -- 2nd ed. (Methods in molecular biology ; 368)

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

842/SMP

Neurochemical Production in Genus *Staphylococcus* by Aromatic Amino Acid Decarboxylase

A. Luqman*[1], P. Stange[2], M. Nega[1], M. T. Nguyen[1], P. Ebner[1], S. Grond[2], F. Götz[1]
[1]*Universität Tübingen, Microbial Genetics, Tübingen, Germany*
[2]*Universität Tübingen, Organic Chemistry, Tübingen, Germany*

Many studies revealed the existence of decarboxylase in bacteria, which make them be able to produce neurochemical. Some other studies also reported that *Staphylococcus* can also produce neurochemical although the responsible genes have not been known yet. We discovered a gene, we named it staphylococcal aromatic amino acid decarboxylase (*sadA*), that encodes the decarboxylase which can convert tryptophan, phenylalanine, and tyrosine into tryptamine, phenylethylamine, and tyramine respectively which are also known as neuromodulator. *sadA* is present in some *Staphylococcus* species but its distribution is not really understandable. Our further investigations revealed that the production of these neurochemicals is not controlled by *agr* system. Besides, the produced neurochemicals also have some inhibition effect on the growth of gram-negative bacteria (*Serratia marcescens* and *Pseudomonas aeruginosa*) at a certain concentration. Insect infection model also confirmed that the produced neurochemicals contribute to the pathogenicity of neurochemical-producing *Staphylococcus*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

843/SMP

Antibiotic acyldepsipeptides vary in activating ClpP from different bacteria

N. Silber*[1], P. Sass[1], H. Brötz-Oesterhelt[1]
[1]*Interfakultäres Institut für Mikrobiologie und Infektionsmedizin Tübingen, Tübingen, Germany*

ADEPs belong to a new class of acyldepsipeptide antibiotics that act via a yet unprecedented mechanism - the dysregulation of the caseinolytic protease Clp, a complex consisting of a proteolytic core, ClpP, that is flanked by corresponding Clp-ATPases. Protease activity of Clp is tightly controlled by Clp-ATPases that bind to distinct hydrophobic pockets of the barrel-shaped ClpP tetradecamer and thread the protein substrates into the degradation chamber. ADEPs compete with and displace the Clp-ATPases from ClpP, thereby preventing its physiological functions [1,2]. Additionally, ADEP binding leads to the enlargement of the entrance pore to the degradation chamber of ClpP [3,4], allowing the degradation of the unfolded model substrate casein as well as nascent polypeptides at the ribosome [1,2]. Treatment with low ADEP concentrations, close to the MIC, resulted in an inhibition of cell division followed by filamentation of rod-shaped bacteria like *Bacillus subtilis* and swelling of cocci like *Staphylococcus aureus*. Further analyses proved that inhibition of cell division was due to the degradation of the essential cell division protein FtsZ by ADEP-activated ClpP [5].
The natural product ADEP1, that is produced by *Streptomyces hawaiiensis* NRRL 15010, is characterized by a lactone core, a phenylalanine linker, and a hydrophobic tail. To improve the activity and stability of ADEP1, different congeners were synthesized [6]. Modifications include the exchange of the N-methylalanine within the pentapeptide ring by pipecolic acid, the decoration of the phenyl ring with two fluorine atoms, and the replacement of the linear conjugated side chain with a shortened cyclohexan-containing moiety.
In vitro experiments suggest that ADEP derivatives differ in their efficacies to activate ClpP depending on the bacterial species. In this project, we aim to investigate this phenomenon to obtain deeper insights into the mechanism of ADEP-binding to ClpP in relation to potential structural differences of ClpP proteins from different species. Using *Escherichia coli* we heterologously expressed and subsequently purified C-terminally His-tagged ClpP and FtsZ from different bacteria. *In silico* analyses, activity tests, and *in vitro* degradation assays using FtsZ will help to identify the reasons for the observed preference of ADEP derivatives to activate ClpP of different bacteria.

References

[1] Brötz *et al*. 2005, Nat. Med. 11: 1082-87
[2] Kirstein *et al*. 2009, EMBO Mol. Med. 1: 37-49
[3] Lee *et al*. 2010, Nat. Struct. Mol. Biol. 17(4): 471-478
[4] Li *et al*. 2010, Chem. Biol. 17(9): 959-969
[5] Sass *et al*. 2011, PNAS 108(42): 17474-17479
[6] Hinzen *et al.* 2006, ChemMedChem 2006, 1, 689-693x.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

844/SMP

Exploiting termite nest microbiomes for antibiotic discovery by using an ultra-high throughput Microfluidics/FACS driven pipeline

J. Glaeser*[1,2], M. Oberpaul*[1,2], M. Nguyen[1,2], T. Stadler[1,2], J. Fracowiak[1,2], R. Plarre[3], P. Hammann[2,4], A. Vilcinskas[1,5]
[1]*Fraunhofer IME, Bioressources, Giessen, Germany*
[2]*Sanofi-Fraunhofer, Natural Products Center, Frankfurt, Germany*
[3]*Bundesanstalt für Materialforschung und -prüfung, Fachbereich 4.1, Berlin, Germany*
[4]*Sanofi-Aventis GmbH, Deutschland, Frankfurt, Germany*
[5]*Justus-Liebig University, Institute for Insect Biotechnology, Giessen, Germany*

Infections with multi-resistant Gram negative pathogens, as *E. coli*., *P. aeruginosa*, *A. baumannii* and *K. pneumonia* are the major threat to our health system. Classic approaches as e.g. the screening of compound libraries or extracts from known antibiotics producing microorganisms failed in the last decades to identify novel chemical scaffolds suitable for the development of antibiotics that reach the market. In order to serve the obvious needs in antibiotics development we selected untapped, easily accessible bio-resources and implemented an ultra-high throughput approach suitable for the discovery of strains producing chemical scaffolds with anti-Gram negative activity. Our approach relies on the hypothesis that insect associated bacteria are likely to produce potent antibiotics with low toxicity to eukaryotic cells to defend their hosts against entomopathogenic microorganisms. Termite nests and guts harbor suitable, highly diverse microbiomes in which bacterial taxa are present that exhibit biosynthetic gene clusters related to antibiotic production. In a first step, the diversity of *Coptotermes* species nest microbiomes was assessed carefully by using 16S rDNA amplicon sequencing and nest material was selected to obtain viable cells by using Nycodenz density gradient centrifugation. In order to analyze the diversity of the culturable termite nest microbiome bacterial cells were encapsulated in agarose microdroplets by an ultra-high throughput microfluidics technique. All microdroplets simultaneously received a small population of GFP tagged Gram negative screening cells for the identification of strains producing anti-Gram negative compounds. Droplets with low or no GFP fluorescence are selected by using fluorescence-activated cell sorting (FACS). After scale-up of droplet microcolonies the activity is validated and compounds underlying the anti-Gram negative activity are identified using UPLC-high resolution MS using an in-house database. Implementation of this pipeline and the design of screening strains allow us to identify thousands of antibiotics producing strains from bio-resources where antibiotics producing bacteria are naturally enriched by screening millions of individual microcolonies that grow separately in microscale droplet compartments.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

845/SMP

Biosynthetic and regulatory analyses of the novel and unique volatile compound sodorifen emitted by *Serratia plymuthica* species

N. Magnus*[1], D. Domik[2], S. von Reuß[3], B. Piechulla[1]
[1]*University of Rostock, Biosciences, Rostock, Germany*
[2]*University of Minnesota Twin Cities, BioTechnology Institute, Minneapolis, United States*
[3]*Université de Neuchâtel, Institut de chimie, Neuchâtel, Switzerland*

A plethora of natural products derive from bacterial origin. Especially the research on new, bioactive secondary metabolites has been expanding largely during the last years since they provide a source for new medicinal products (e.g. antibiotics). Nevertheless, the relevance of small (volatile) compounds as bioactives has been underestimated in the past. Microorganisms can produce a wide variety of such substances called volatile organic compounds (VOCs). For example, the Gram-negative bacterium *Serratia plymuthica* 4Rx13, isolated from the rhizosphere of *Brassica napus* (oilseed rape), is capable of emitting up to 100 different VOCs depending on the cultivation conditions. GC-MS analyses of the volatile bouquet of *S. ply.* 4Rx13 indicated a dominant compound (ca. 50 %) of unknown structure. Subsequently, GC-HRMS and NMR analyses revealed an intriguingly unusual molecule consisting of only carbon and hydrogen, organized in a bicyclic ring system where each carbon atom was substituted with a methyl- or methylene group. This completely new compound was named sodorifen, for which so far no biological function could be assigned. Also, until now only five bacterial strains were found to produce sodorifen, all of them belonging to the species *Serratia plymuthica*. Following a genomic and transcriptomic approach the biosynthesis of sodorifen was elucidated, revealing a biosynthetic gene cluster consisting of four genes. Subsequently, its essential role in sodorifen production was proved by insertion mutagenesis. The obtained results substantiated the hypothesis that sodorifen belongs to the chemical class of sesquiterpenes. Ongoing research is currently performed on the regulatory processes involved in the synthesis and emission of sodorifen. By investigating the expression level of the sodorifen cluster genes in i) different *Serratia* spp. and ii) under varying nutrient conditions a hierarchical regulatory network became apparent. This network involves transcriptional, as well as post-transcriptional mechanisms (including carbon catabolite repression). Moreover, there is evidence that the sodorifen emission is under control of at least one luxI/R-like quorum sensing system. Therefore, our investigations revealed that sodorifen is a highly unusual natural product with a multi-layered regulatory network controlling its production.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

846/SMP

Pseudoxylallemycins A–F, cyclic tetrapeptides with rare allenyl modifications isolated from *Pseudoxylaria* sp. X802 – a competitor of fungus-growing termite cultivars

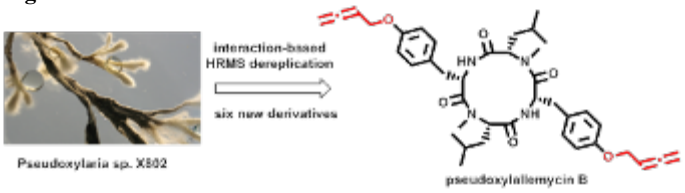
H. Guo*[1], P. Stephan[1], N. Kreuzenbeck[1], M. Garcia-Altares[1], S. Otani[2], H. M. Dahse[1], C. Weigel[1], M. Poulsen[2], C. Beemelmanns[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany*
[2]*University of Copenhagen, Department of Biology, Copenhagen, Denmark*

Introduction: Termites of the subfamily Macrotermitinae live in an obligate symbiosis with a specialized fungal cultivar *Termitomyces* sp. (Basidiomycotina),[i],[ii] which is cultivated on faecal deposits of dead pre-digested plant material (the fungus comb). *Pseudoxylaria*, a subgenus within the genus *Xylaria* (Ascomycota: Xylariaceae), is frequently found on deteriorating comb material and is presently considered a stowaway fungus, waiting as a substrate specialist and opportunistic weed until conditions are favourable for outcompeting *Termitomyces*.[iii]
Objectives: Based on the competitive and/or antagonistic behaviour, we hypothesized that termite-associated *Pseudoxylaria* may produce biologically active small molecules upon exposure to other fungi from the same ecological niche.
Materials & methods: Using a combination of fungus-fungus pairing assays and a HRMS-based dereplication strategy, biologically active small molecules are identified by NMR spectroscopy and Marfey"s analysis.
Results: Six new cyclic tetrapeptides named pseudoxylallemycins A–F were identified from *Pseudoxylaria* sp. X802.[iv] Pseudoxylallemycins B–D possess a rare and chemically accessible allene moiety, which is amenable for synthetic modifications. Pseudoxylallemycins A–D show antimicrobial

activity against gram-negative human-pathogenic *Pseudomonas aeruginosa* and antiproliferative activity against HUVEC and K-562 cell lines.
Conclusion: This discovery not only reveals the chemical potential of hidden niche--fungus growing termites associated microbe, provides candidates for drug discovery, but also an effective strategy to understand the chemical basis of microbial interaction inside this ecological niche.

References
[1] Sands, W. A. In *Biology of Termites*; Krishna, K., Weesner, F. M., Eds.; Academic Press: New York, 1969, chapter 16, pp. 495–524.
[2] Aanen, D. K.; de Fine Licht, H. H.; Debets, A. J. M.; Kerstes, N. A. G.; Hoekstra, R. F.; Boomsma, J. J. *Science* 2009, *326*, 1103–1106.
[3] Visser, A. A.; Kooij, P. W.; Debets, A. J. M.; Kuyper, T. W.; Aanen, D. K. *Fungal Ecol.* 2011, *4*, 322–332.
[4] Guo, H.; Kreuzenbeck, N. B.; Otani, S.; Garcia-Altares, M.; Dahse, H.-M.; Weigel, C.; Aanen, D. K.; Hertweck, C.; Poulsen, M.; Beemelmanns, C. *Org. Lett*. 2016, *18*, 3338–3341.

Figure 1



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

847/SMP
Functional and chemical analysis of Actinobacteria associated with fungus-growing termites
R. Benndorf*[1], H. Guo[1], E. Sommerwerk[1], C. Weigel[1], S. Otani[2], M. Poulsen[2], C. Beemelmanns[1]
[1]*Leibniz Institute for Natural Product Research and Infection Biology e.V. Hans-Knöll-Institute (HKI), Chemical Biology of Microbe-Host Interactions, Jena, Germany*
[2]*University of Copenhagen, Department of Biology, Copenhagen, Denmark*

Introduction: *Macrotermitinae* (fungus-growing termites) cultivate a mutualistic food fungus (*Termitomyces* sp.) for nourishment in so called "fungus gardens",[1] a nutrient-rich environment prone to exploitation. Symbiotic and associated bacteria are assumed to play a major role in the defense of the fungus garden by secretion of antimicrobial small molecules, which selectively target garden parasites and other invading species.[2]
Objectives: We focus on the functional, genomic and chemical analysis of Actinobacteria associated with fungus growing termites to study their role as defensive symbionts.
Methods: Using different culture-depended methods, we first isolated Actinobacteria from various different insect body parts. Using the obtained isolates, we then performed different activity assays to investigate the (selective) inhibition of fungal garden parasites. Strains with particular high antifungal activity were selected for further chemical analysis. Dereplication was performed using MS-based techniques. The produced antimicrobial secondary metabolites were analyzed and characterized using HPLC/LC-HRMS/NMR. In parallel, we sequenced selected isolates to investigate the strains on genomic level.
Results: Using selective culture medium, more than 100 different members of Actinobacteria phylum were isolated from different body parts of termite workers and the termite nest.[3] Extracts of the bacteria culture were tested against known human pathogens showing a high antimicrobial activity. Pairing challenging assays of Actinobacteria extracts showed activity against co-isolated and antagonistic fungi, such as *Pleosporales sp.* Several new metabolites were identified and characterized. We are now developing a culture-independent approach to describe and analyze the antimicrobial environment, which antagonist"s meet when entering the nest.
Conclusion: Microbial symbionts and commensals most likely contribute to the fungus garden homeostasis by secretion of antimicrobial compounds.

References
[1] a) Aanen, D. K.; Eggleton, P.; Rouland-Lefèvre, C.; Guldberg-Frøslev, T.; Rosendahl, S.; Boomsma, J. J. *Proc. Natl. Acad. Sci. U. S. A*. 2002, *99*, 14887–14892; b) Poulsen, M.; Hu, H.; Li, C.; Chen, Z.; Xu, L.; Otani, S.; Nygaard, S.; Nobre, T.; Klaubauf, S.; Schindler, P. M.; Hauser, F.; Pan, H.; Yang, Z.; Sonnenberg, A. S. M.; de Beer, Z. W.; Zhang, Y.; Wingfield, M. J.; Grimmelikhuijzen, C. J. P.; de Vriese, R. P.; Korb, J.; Aanen, D. K.; Wang, J.; Boomsma, J. J.; Zhang, G. *Proc. Natl. Acad. Sci. U. S. A*. 2014, *111*, 14500–14505.
[2] a) Beemelmanns C., Guo H., Rischer M., Poulsen M. *Beilstein J Org Chem* 2016 12, 314-327; b) Ramadhar, T. R; Beemelmanns, C.; Currie C. R.; Clardy J. *J Antibiot (Tokyo)* 2014**,** 67(1), 53-58.
[3] Visser, A. A.; Nobre, T.; Currie, C. R.; Aanen, D. K.; Poulsen, M. *Microb. Ecol.* 2012, *63*, 975–985.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

848/SMP
Deciphering the Melleolide Biosynthesis Pathway of *Armillaria gallica*
C. McELroy*[1], S. Jennewein[1]
[1]*Fraunhofer IME , Industrial Biotechnology, Aachen, Germany*

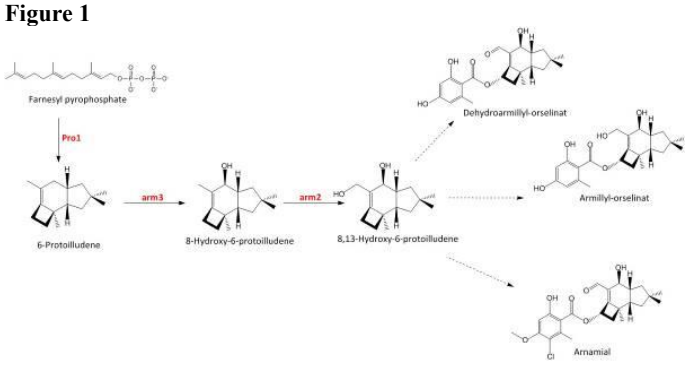
Increased infections from antibiotic resistant bacteria has promoted the search for novel compounds possessing potent antimicrobial activities with novel modes of action. One such group of candidate compounds are the melleolides. Over 50 melleolides have been isolated to date from mycelial cultures of the Homobasidiomycetes genus *Armillaria*. Respective cytotoxicity and antimicrobial activity is highly dependent upon specific melleolide structure. However, despite the large variety identified, very little is actually known with regards to the melleolide biosynthesis pathway.
Furthermore, complex regiochemistries encumber chemical synthesis of the most potent of these identified compounds. Thus the elucidation of this pathway is necessary for melleolide based antimicrobial development. Fortunately, the location of the biosynthesis cluster within the genome of *Armillaria gallica* has been identified (Jennewein and Engels, 2015), within which the terpene synthase responsible for catalyzing the first committing step has been well characterized (Engels et al., 2011). This provides an excellent foundation for uncovering the rest of the pathway which comprises of many more proteins i.e. monooxygenases, dehydrogenases, polyketide synthases etc.
The aim therefore, is to use genomic and transcriptomic data to identify enzyme candidates which will subsequently be cloned and expressed within an easily metabolically engineered and fermentable host such as *Saccharomyces cerevisiae* host. Once created, these strains can be used for enzyme characterization within two experimental strategies. The first relies upon feeding these strains with [3H]-6-protoilludene (the radioactively-labelled product of the terpene synthase) in order to accurately determine substrate conversion. Secondly, strain creation for the autonomous production of novel pathway intermediates, which can then be purified and analyzed via GC/MS (gas chromatography mass

spectrometry) and NMR (nuclear magnetic resonance) microscopy. The heterologous expression of protoilludene synthase and its subsequent purification has facilitated its use for the biocatalysis of [3H]-6-protoilludene which is being used to elucidate further pathways steps. Ultimately, new pathway intermediates permit the further elucidation of this secondary metabolite pathway.

References

[1] Engels, B., Heinig, U., Grothe, T., Stadler, M. & Jennewein, S. 2011. Cloning and characterization of an Armillaria gallica cDNA encoding protoilludene synthase, which catalyzes the first committed step in the synthesis of antimicrobial melleolides. *J Biol Chem,* 286**,** 6871-8.
[2] Jennewein, S. & Engels, B. 2015. Melleolide-biosynthesis gene cluster and its application. WO2015104051A1.

Figure 1



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

849/SMP

Structural prerequisites for enzymatic flavin-N5-oxide formation

R. Saleem Batcha*[1], R. Teufel[1]
[1]*University of Freiburg, Center for Biological Systems Analysis (ZBSA), Freiburg, Germany*

The intriguing reaction of the ubiquitous flavoproteins with molecular oxygen has been intensively studied for many decades, yet many open key questions remain to be answered. The flavin-dependent monooxygenases are among the most comprehensively studied enzymes that accomplish a variety of redox reactions such as the monooxygenation of organic substrates. Our understanding of the enigmatic reaction of enzyme-bound reduced flavin with oxygen is incomplete, as underlined by recent studies on the biosynthesis of the bacterial polyketide antibiotic enterocin. Here, the flavin-dependent monooxygenase EncM acts as the key tailoring enzyme by oxygenating the enterocin precursor with an unusual flavin-N5-oxide intermediate. Before then, it was presumed that all flavin-dependent monooxygenases employ the flavin-C4a-hydroperoxide as the oxygenating species. We aim to identify the structural prerequisites for the generation of this novel flavin redox state and investigate EncM variants as well as homologous flavoproteins (native and variants), which do not naturally form the flavin-N5-oxide. With the help of the crystal structure of EncM and *in silico* docking methods, a model for the interaction of O2-binding pocket was predicted and various EncM mutants were generated. Moreover, protein crystals suitable for X-ray crystallographic studies were obtained for several EncM mutants allowing the assessment of the structural changes. In addition, we used an X-ray crystallographic technique with pure oxygen under high pressure on EncM. We could visualize the oxygen binding mode of EncM and thus acquire convincing evidence for a dedicated oxygen binding pocket close to the flavin cofactor. We anticipate to gain significant understanding of the structural prerequisites and mechanistic basis for flavin-N5-oxide formation, which may ultimately allow to fine-tune the oxygen reactivity of flavoproteins through rational engineering.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

850/SMP

Investigation of the enzymatic formation of the griseorhodin A pharmacophore

B. Frensch*[1], R. Teufel[1]
[1]*University of Freiburg, Center for Biological Systems Analysis (ZBSA) and Faculty of Biology in Collaboration with Jörn Piel, ETH Zürich , Freiburg, Germany*

Griseorhodin A is one of the most heavily oxidized bacterial polyketides known that can be isolated from *Streptomyces* sp.. It is a member of the rubromycin family - a group of extensively modified aromatic polyketides that inhibit HIV reverse transcriptase and human telomerase. The highly unusual spiroketal pharmacophore of the rubromycin polyketides was shown to be required for bioactivity.[1,2] In the course of gene knock-out experiments with a heterologous producer, the polycyclic aromatic compound collinone could be isolated from a mutant strain. Collinone is highly similar to griseorhodin A, but lacks the spiroketal moiety that is likely formed in subsequent pathway steps via extensive enzymatic oxidative rearrangement of the collinone carbon skeleton. These unprecedented tailoring steps may be catalyzed by the predicted FAD-dependent enzymes GrhO1, GrhO5 and GrhO6.[1,2] We aim to reconstitute the spiroketal-forming steps *in vitro* to gain a detailed understanding of the individual enzymatic reactions and catalytic mechanisms. Initial studies show the promising conversion of collinone into various compounds, which are currently characterized by MS and NMR. The biosynthetic prowess of the enzymes will be further investigated with the goal to incorporate the spiroketal moiety into other aromatic compounds.

References

[1] A. Li, J. Piel, *Chemistry and Biology* 2002, *9*, 1017−1026.
[2] Z. Yunt, K. Reinhardt, A. Li, M. Engeser, H.-M. Dahse, M. Gütschow, T. Bruhn, G. Bringmann, J. Piel, *J. Am. Chem. Soc.* 2009, *131*, 2297−2305.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Symbiotic Interactions (FG SI)

851/SIP

Metagenome, metatranscriptome and exo-proteome analyses identify molecular keys and main bacterial players involved in chlorophyta and charophyta microalgae-bacteria interactions

I. Krohn-Molt*[1], M. Alawi[2], D. Indenbirken[2], L. Burkhardt[2], M. Thieß-Jünger[3], A. Wiegandt[4], K. Förstner[5], A. Grundhoff[2], J. Kehr[3], A. Tholey[4], W. R. Streit[1]

[1]*Universität Hamburg, Mikrobiologie und Biotechnologie, Hamburg, Germany*

[2]*University Medical Center Hamburg-Eppendorf, Bioinformatics Service Facility, Hamburg, Germany*

[3]*University of Hamburg, Biocenter Klein Flottbek, Molecular Genetics, Hamburg, Germany*

[4]*Christian-Albrecht-University, Division of Systematic Proteome Research & Bioanalytics, Kiel, Germany*

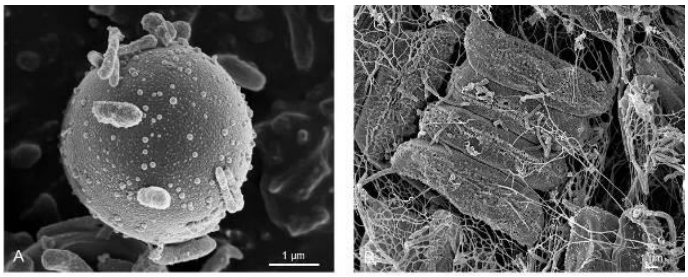
[5]*University of Würzburg, Core Unit Systems Medicine, Würzburg, Germany*

Microalga belonging to the genus of the Chlorophyta and the Charophyta are considered to be evolutionary early plant lines. Due to these phylogenetic properties makes them interesting models to study "eukaryotic-bacteria" interactions at an evolutionary very early level. By analyses via 16S rRNA amplicon sequencing we identified a maximum of 22 bacterial operational taxonomic units (OTUs) associated with different microalga. The majority of bacteria identified were affiliated with the phyla of the α-, β- Proteobacteria, and the Bacteroidetes. Thereby we observed patterns of host specific colonization for members of the Chlorophyta and Charaophyta. Therefore, here we report on insight into the microbiomes of two Chlorophyta (*Chlorella saccharophila* and *Scenedesmus quadricauda*) and one Charophyta (*Micrasterias crux-melitensis*). Our detailed metagenome analyses via HiSeq 2500 from Illumina resulted in an overall assembly of 174 Mbp for the bacterial metagenome of *Chlorella saccharophila*, a length of 162 Mbp for the bacterial metagenome of *Scenedesmus quadricauda*, and 268 Mbp of assembled DNA for *Micrasterias crux-melitensis*. A comprehensive pathway analyses suggested between 236,273 and up to 349,091 protein coding genes for each microalgae bacterial community. Interestingly, bining analyses indicated a prevalence of α-Proteobacteria in the Chlorophyta and a prevalence of the Bacteroidetes for the Charophyta. RNAseq data further implied that the microbiomes differed largely with respect to the global transcription patterns. To calculate the gene expression level of the different bacterial communities, a minimum of 10.0 Reads Per kb per Million reads (RPKM) was used. The RNAseq data resulted in a total number of genes with sufficiently high RPKM calculation of 5,198 genes for the bacterial community of *Chlorella saccharophila*, 7,603 genes for the microbiome of *Scenedesmus quadricauda*, and 7,764 genes for the associated bacteria of *Micrasterias crux-melitensis*. However, a shared and common set of 867 genes was identified and transcribed in each alga microbiome. In line with the phylogenetic analyses of the most abundant genome bins the majority of the 1,000 most strongly transcribed genes in the Chloropyhta derived from the *Sphingomonadales.* In the Charophyta microbiome highest transcribed genes originated from the *Sphingobacteriales*. Additional exo-proteome analyses of the *Scenedesmus quadricauda* microbiome implied that besides the *Sphingomonadales,* uncultivated bacteria and *Mesorhizobia* play a pivotal role. Genes and proteins, that were most frequently observed were elicitors involved in challenging plant innate immune system. This finding potentially implies an evolutionary early development of protective mechanisms.

figure legend:

FIGURE 1: A) Scanning electron micrograph (SEM) of *Chlorella saccharophila* (MZCH 10155), **B)** *Scenedesmus quadricauda* (MZCH 10104). The scale bars of 1µm are indicated in the images. (REM LEO 1525, 5.00 kV).

Figure 1



Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION
Synthetic Microbiology and Biotechnology (FG SMB)

852/SMBP

Construction and analysis of recombinant isobutanol-producing *Clostridium ljungdahlii* strains

S. Weitz*[1], F. Bengelsdorf[1], P. Dürre[1]

[1]*Universität Ulm, Insitut für Mikrobiologie und Biotechnologie, Ulm, Germany*

Introduction and objectives: The current method used for the industrial isobutanol production is the carbonylation of propylene, which is produced from fossil fuels. As a potential alternative, several recombinant strains (of *Escherichia coli, Corynebacterium glutamicum, Bacillus subtilis, Saccharomyces cerevisiae, Ralstonia eutropha*) were constructed to produce isobutanol using sugars as carbon sources.

Waste gas streams containing CO2, CO and H2 can be used as carbon and energy source for autotrophic bacteria to circumvent food competing substrates. Autotrophic acetogenic bacteria carry out the Wood-Ljungdahl pathway to reduce CO2 + H2, and / or CO to the central intermediate acetyl-CoA which is further converted to byproducts acetate and ethanol. Acetyl-CoA is also metabolized to pyruvate, which is afterwards converted, among others, to valine.

Ketoisovalerate, the precursor of valine, can be converted to isobutanol using a ketoisovalerate decarboxylase (KIVD) and an alcohol dehydrogenase (ADH). The ability to convert ketoisovalerate to isobutanol should be introduced to the acetogenic autotrophic bacterium *Clostridium ljungdahlii* by the heterologous expression of KIVD and ADH.

Materials and methods: The genes *kivD* of *Lactococcus lactis* and *adhA* of *C. glutamicum* were chosen for the heterologous expression and*, ilvC, ilvD,* and *alsS* of *C. ljungdahlii* were chosen for the homologous overexpression to establish the isobutanol production in *C. ljungdahlii*. These genes were assembled and cloned into the vector pMTL83151.

The protocol of Leang *et al*., (2013) was used for the transformation of *C. ljungdahlii*. The growth experiment was performed in modified medium (Tanner, 2007) with a pH of 6, 40 mM fructose, 20 mM ketoisovalerate and the respective controls. Products were analyzed by gas chromatography and high performance liquid chromatography.

Results: The mentioned genes were cloned into the plasmid pMTL83151 resulting in the production plasmid pMTL83151_KAIA, subsequently the transformation of plasmid

pMTL83151_KAIA in *C. ljungdhlii* was carried out resulting in the recombinant strain CLJU[pMTL83151_KAIA]. In the following growth experiment, CLJU[pMTL83151_KAIA] produced ethanol and acetate and utilized all added fructose. By adding 20 mM of ketoisovalerate to the medium, *C. ljungdahlii* pMTL83161_KAIA showed a slower growth, but reached the same final optical density compared to the approach without ketoisovalerate. Furthermore, CLJU[pMTL83151_KAIA] produced isobutanol, ethanol, and acetate. Ketoisovalerate and fructose were completely consumed. In both growth experiments the adjusted pH of 6 decreased to 5.
Conclusion: *C. ljungdahlii* is able to produce isobutanol when the genes *kivd, adhA, ilvC, ilvD,* and *alsS* are overexpressed and ketoisovalerate is added to the medium. The isobutanol production needs to be further improved and also tested under autotrophic growth conditions.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

853/SMBP
Innovative downstream processing techniques for the production of recombinant cytokines based on membrane adsorbers
A. Krüger*[1], H. Piascheck[1], V. Nölle[2], U. Odenthal[2], L. Villain[3], S. Beutel[4], T. Scheper[4], G. Antranikian[1]
[1]*Hamburg University of Technology, Institute of Technical Microbiology, Hamburg, Germany*
[2]*Miltenyi Biotec GmbH, Bergisch Gladbach, Germany*
[3]*Sartorius Stedim Biotech GmbH, Göttingen, Germany*
[4]*Leibniz University of Hannover, Institute of Technical Chemistry, Hannover, Germany*

Introduction: Cytokines are a heterogeneous group of small proteins (5-20 kDa) playing a key role in proliferation or differentiation of target cells or immune responses. Their *in vitro* application ranges from cellular immune therapies to artificial tissues and organs. Among the cytokines, bone-morphogenetic proteins (BMPs) are growth factors belonging to the cysteine-knot family, which are important for the induction of bone and cartilage formation. Therefore, they are clinically applied during fracture treatment and regenerative bone therapies [1].
Objectives: The goal of the study was the development and upscaling of a fermentation and downstream processing (DSP) protocol for the production and purification of human bone-morphogenetic protein-2 (BMP-2).
Materials & methods: Inclusion bodies obtained from fermentation of a recombinant *Escherichia coli* production strain were isolated and solubilized, followed by refolding and dimerization of BMP-2 according to previous protocols [2,3]. Subsequent separation of the BMP-2 dimer from remaining monomeric fractions was carried out with a membrane adsorber.
Results: A fed-batch fermentation set-up was successfully applied and scaled-up for the efficient production of BMP-2 inclusion bodies. Various refolding and dimerization conditions were tested to increase the yield of the BMP-2 dimer. The optimized protocols were then applied for a scale-up of the downstream process from 1 ml to 600 ml with subsequent purification of the BMP-2 dimer applying membrane adsorber techniques.
Conclusion: The production of human BMP-2 with *E. coli* was optimized and a robust and reproducible DSP based on membrane adsorbers was developed and scaled-up to industrially relevant volumes.

References
[1] Gieseler G, Pepelanova I, Stuckenberg L, Villain L, Nölle V, Odenthal U, Beutel S, Rinas U, Scheper T (2016) Purification of bone morphogenetic protein-2 from refolding mixtures using mixed-mode membrane chromatography. Appl Microbiol Biotechnol. doi:10.1007/s00253-016-7784-1.
[2] Vallejo LF, Rinas U (2004) Optimized procedure for renaturation of recombinant human bone morphogenetic protein-2 at high protein concentration. Biotechnol Bioeng 85(6):601–609. doi:10.1002/bit.10906.
[3] Vallejo LF, Brokelmann M, Marten S, Trappe S, Cabrera-Crespo J, Hoffmann A, Gross G, Weich HA, Rinas U (2002) Renaturation and purification of bone morphogenetic protein-2 produced as inclusion bodies in high-cell-density cultures of recombinant *Escherichia coli*. J Biotechnol 94(2):185–194. doi:10.1016/s0168-1656(01)00425-4.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

854/SMBP
Protein engineering to improve biocatalysts for novel biotechnological applications
M. Maier*[1], K. S. Rabe[1]
[1]*Karlsruhe Institute of Technology, Institute for Biological Interfaces 1, Eggenstein-Leopoldshafen, Germany*

Enzymes from mesophilic sources often deal poorly with harsher reaction conditions which can be encountered in industrial processes, especially high temperatures. As a means to deal with these challenges, protein engineering can be used to improve existing or enable new processes in biotechnology, leading to new bio-based products or applications.
We recently reported the use of a thiamin-pyrophosphate dependent decarboxylase in an extremophile host for the production of isobutanol.[1] Using a thermophile host, we expect to overcome the physical limitations of mesophile organisms due to their physiological bias. However, the production of isobutanol at elevated temperatures (>50°C) was hampered due to the limited stability of the enzyme. Thus we used directed evolution (computational and random approaches) to improve the thermostability of the decarboxylase while maintaining its activity. The screening assay was based on the consumption of NADH thus correlating the activity of the particular KIVD variants with an optical readout. In order to further investigate the stability and activity of improved variants in a more direct fashion, HPLC analysis was performed quantifying the product formation at 60°C. This way we were able to find single amino acid mutations which contributed markedly to an enhanced stability without compromising the enzymatic activity. Preliminary experiments also indicate activity of the engineered enzyme variants in *Geobacillus* spec. at elevated temperatures >50°C.

References
[1] P. P. Lin, K. S. Rabe, J. L. Takasumi, M. Kadisch, F. H. Arnold, and J. C. Liao, 'Isobutanol Production at Elevated Temperatures in Thermophilic Geobacillus thermoglucosidasius', Metab Eng, 24C (2014), 1-8.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

855/SMBP

Caged compounds as light sensitive switches for the production of valuable metabolites in biotechnology

F. Hilgers*[1], D. Binder[1], C. Bier[2], F. Hogenkamp[2], A. Loeschcke[1], J. Pietruszka[2], K. E. Jaeger[1,3], T. Drepper[1]
[1]*Heinrich Heine University at the area of Forschungszentrum Jülich, Institute of Molecular Enzymtechnology, Jülich, Germany*
[2]*Heinrich Heine University at the area of Forschungszentrum Jülich, Institute of Bioorganic Chemistry, Jülich, Germany*
[3]*Forschungszentrum Jülich, Institute of Bio- and Geosciences (IBG-1) , Jülich, Germany*

Optogenetic tools exploit the diverse properties of the environmental factor light in order to achieve a non-invasive and immediate control of cellular functions. For example, light-controlled switches allow to facilitate a precise coordination of gene expression in the context of complex biosynthetic networks and are therefore suitable for many different biotechnological applications. [1]
Since effective biosynthesis of valuable secondary metabolites in heterologous hosts is often hampered by inefficient metabolite fluxes, this work aimed to achieve an exact and straightforward triggering of complex gene clusters by using light-sensitive photocaged compounds such as caged arabinose [2] or caged galactose [3].
To demonstrate the applicability of caged carbohydrates as light-sensitive inducers for gene expression in *E. coli*, expression of the heterologous violacein gene cluster was gradually induced upon increasing UV-A light intensities by means of photocaged arabinose. Since unbalanced expression of *vioC* and *vioD* results in the formation of by-product deoxyviolacein, the violacein pathway was subsequently engineered to exclusively form one of the two bisindole derivatives. The modulation of the original violacein gene cluster will thus finally enable the evaluation of light-controlled production of selected pathway intermediates.
Optogenetic tools proved to be a versatile and valuable approach to enable precise and straightforward triggering of different microbial production processes with remarkable spatiotemporal resolution in a non-invasive fashion. Henceforth, they might allow a precise timing of complex pathways to accumulate particular intermediates by means of multimodal optogenetic control in near future.

References

[1] Drepper, T., Krauss, U., Meyer zu Berstenhorst, S., Pietruszka, J., and Jaeger, K.-E. (2011). Applied microbiology and biotechnology, 90(1), 23–40.
[2] Binder, D., Bier, C., Grünberger, A., Drobietz, D., Hage-Hülsmann, J., Wandrey, G., Büchs, J., Kohlheyer, D., Loeschcke, A., Wiechert, W., Jaeger, K.-E., Pietruszka, J., and Drepper, T. (2016). ChemBioChem, 17(4), 296–299.
[3] Bier, C., Binder, D., Drobietz, D., Loeschcke, A., Drepper, T., Jaeger, K.-E. and Pietruszka, J. (2016). Synthesis, *accepted*. DOI: 10.1055/s-0035-1562617

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

856/SMBP

Immobilization of Biocatalysts for Microfluidic Reaction Cascades

T. Peschke*[1], K. Rabe[1], C. Niemeyer[1]
[1]*Karlsruhe Institute of Technology (KIT, Institute for Biological Interfaces (IBG 1), Eggenstein-Leopoldshafen, Germany*

Nature has evolved a highly efficient metabolic network in the form of fine-tuned enzymatic multistep reactions that ensure life. Mimicking these naturally optimized systems in a microfluidic system, could enable the biotechnological accessibility of molecules, which are not readily available by conventional approaches. [1] One key aspect when developing an artificial microfluidic biocatalytic cascade concerns the immobilization of accessible and active biocatalysts. Usual approaches use a biocatalyst which is non-specifically immobilized (e.g. physically adsorbed, chemically cross-linked or entrapped) thus leading to random orientations with respect to the solid support. These non-specific immobilization techniques often decrease the overall biocatalytic activity.
The aim of this project is to establish immobilization strategies which are biorthogonal, show a low or no dissociation constant and high binding specificity. As a proof of concept, a one-pot enzymatic three-enzyme cascade was selected, which has recently been described by Skoupi *et al.,* 2015 [2] for the synthesis of *meso* diols. This was then optimized for the processes required for a compartmentalised microfluidic reactor.
To this end, purified enzymes as well as whole cells are site-specifically immobilized on surfaces such as superparamagnetic microparticles. The functionalized particles are loaded in a microfluidic packed bed reactor, [3] thereby enabling the fluidic coupling of two consecutive biocatalytic conversions. Using this approach, the fluidic production of the *meso* diol from a diketone using R- and S-specific ketoreductases was achieved in high yields (82%) with excellent stereoselectivities (e.r. >99:1 and d.r. >91:9).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

857/SMBP

***Rhodobacter capsulatus* as alternative microbial platform for terpenoid production**

K. Troost*[1], A. Loeschcke[1], K. E. Jaeger[1,2], T. Drepper[1]
[1]*Institut für molekulare Enzymtechnologie (IMET) /Heinrich-Heine-Universität Düsseldorf, Jülich, Germany*
[2]*Institut für Bio- und Geowissenschaften IBG-1/Forschungszentrum Jülich, Jülich, Germany*

Terpenoids belong to the largest group of natural products, which are characterized by a very high functional and structural variety. Many essential oils containing terpenoids have therapeutical effects, e.g. stimulating the blood flow or exhibiting anti-microbial, -fungal, -viral or -cancer activities. Therefore, they are valuable target compounds for the pharmaceutical industry. Since the production of terpenoids in their natural producers (i.e. mostly plants) is not very cost-efficient and convenient, the heterologous production in microorganisms has emerged as a promising alternative. Considering the required precursor supply for the production of terpenoids, it is useful to employ production organisms, which are natural terpenoid producers, like *Rhodobacter capsulatus*. The facultative phototrophic bacterium is capable of naturally synthesizing the tetraterpene carotenoids spheroidene and spheroidenone in high amounts providing the terpenoid precursors through the DXP- (1-deoxy-D-xylulose-5-phosphate) pathway.
We aim here to evaluate the suitability of the purple bacterium *R. capsulatus* for the production of terpenoids using the patchoulol synthase from *Pogostemon cablin* as an example. The production of patchoulol was comparatively evaluated using the expression vectors pRhokHi-2 (weak, P*aphII*, constitutive expression), pRhofHi-2 (weak, P*fru*, inducer: fructose), pRhotHi-2 (strong, PT7, inducer: fructose), and pRhon5Hi-2 (P*nif*, very strong, induction: NH4+ limitation). Moreover, co-expression of (i) DXP pathway-limiting enzymes like IspA, Dxs, Idi, (ii) the MVA pathway genes from *Paracoccus zeaxanthinfaciens* and (iii) using different mutant strains of *R. capusulatus* was tested to increase final product yields. For the analysis, phototrophically grown cultures were overlayed with n-dodecane to entrap patchoulol and enable straightforward sampling for GC-MS analysis.
We could show that the P*nif*-expression system is the best choice for effective patchoulol production. Furthermore, the co-

expression of the DXP pathway-limiting enzymes as well as the deletion of the *crtE* gene significantly improves product formation. Finally, the best product yield was reached when the MVA pathway genes were co-expressed.
Due to its specific metabolic capacity, *R. capsulatus* appears to be an especially suitable production host for sesquiterpenoid production. The comparative analysis demonstrated that the promoter strength of the expression system as well as the efficiency of isoprenoid precursor biosynthesis play important roles for product formation of terpenoids in *R. capsulatus*.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

858/SMBP
An esterase-based reporter system to analyze protein production in extremophile hosts
F. Engel*[1], L. Arnold[1], K. S. Rabe[1]
[1]*Karlsruhe Institute of Technology, Institute for Biological Interfaces 1, Eggenstein-Leopoldshafen, Germany*

Recently there has been a growing interest in the use of non-standard (not *E. coli* or Yeast) organisms in biotechnology. However, in order to understand and engineer such organisms and to establish them as chassis for synthetic biology and biotechnology, new tools have to be developed in order to monitor processes on the molecular level and inside the cell. Especially the detailed understanding and optimization of promoters in order to fine tune protein expression requires quantification on the single cell level. The standard reporter gene GFP and also its more stable derivatives displayed no detectable fluorescence when analyzed *in vivo* in *Thermus thermophilus* HB27 at elevated temperatures in our hands.
We thus developed a thermostable esterase into a reporter protein. The protein is active at a broad range of environmental conditions, it is monomeric, does not need maturation or cofactors and can be applied as an *in vivo* reporter. Furthermore, it offers the advantage of signal amplification due to its enzymatic activity, which results in a low limit of detection.
We first purified the enzyme and analyzed its kinetic parameters regarding six different fluorogenic esterase substrates. Subsequently we cloned and tested the system in *Thermus thermophilus* HB27 as well as *E. coli*. Intravital whole cell measurements now open the way to engineer promoters inside extremophile hosts, since a high-throughput selection of improved mutants can be performed at the single cell level. We will show examples of different promoters and their use in *T. thermophilus*. In general the proposed reporter protein will enable the molecular analysis of fundamental biological questions regarding the lifestyle of thermophiles and other extremophiles and open the way to utilizing these organisms as whole cell catalysts.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

859/SMBP
Functionalization of bacterial nanoparticles by genetic engineering and surface display of peptides and reporter enzymes
F. Mickoleit*[1], D. Schüler[1]
[1]*University of Bayreuth, Dept. Microbiology, Bayreuth, Germany*

One intriguing example for a biomineralization process is the formation of magnetosomes in the magnetotactic alphaproteobacterium *Magnetospirillum gryphiswaldense*. Magnetosomes are membrane-enveloped magnetic nanocrystals of Fe_3O_4 that are aligned in regular intracellular chains which serve as a geomagnetic field sensor [1]. Due to their unprecedented properties (high crystallinity, strong magnetization, uniform shapes and sizes), magnetosomes have an enormous potential for biomedical and biotechnological applications. Both their crystal morphologies and the composition of the enveloping membrane can be manipulated by genetic means, allowing a controlled functionalization of the magnetosome surface by genetic engineering. For that purpose, a versatile and diverse genetic "toolkit" for the generation of "smart" multifunctional magnetic nanoparticles with several tailored properties is being created.
Using an optimized expression system [2], several of the most abundant proteins of the magnetosome membrane (MamC/A/F/G) were tested as potential membrane anchors for the magnetosomal display of reporter proteins, including eGFP as well as the β-glucuronidase GusA. Magnetosome-expressed GusA followed Michaelis-Menten kinetics, and specific activities were comparable to the non-immobilized enzyme. GusA magnetosomes turned out to be reusable and resistant against repeated freeze-thawing steps. Moreover, the expression of multiple arrays of GusA led to a significant increase of the particle-displayed enzymatic activity.
In addition, shell size and properties were modified by the expression of artificial peptides. These functional extensions significantly increased the hydrodynamic diameter of the magnetosome shell and altered the surface charge. As an alternative to our inorganic coatings [3], strategies are investigated to generate organic / polymeric porous shells that are expected to improve biocompatibility and provide tuneable characteristics.

References
[1] Jogler C., Schüler D. (2009) *Annu. Rev. Microbiol*. 63:501-521.
[2] Borg S., Hofmann J., Pollithy A., Lang C., Schüler D. (2014) *Appl. Environ. Microbiol.* 80:2609-2616.
[3] Borg S., Rothenstein D., Bill J., Schüler D. (2015) *Small* 11:4209-4217.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

860/SMBP
Exploring a new potential protein synthesis inhibitor in *Streptomyces aureofaciens* TÜ1-01
F. Handel*[1], W. Wohlleben[1], Y. Mast[1]
[1]*Universität Tübingen; Interfakultäres Institut für Mikrobiologie und Infektionsmedizin , Mikrobiologie/Biotechnologie, Tübingen, Germany*

Antibiotics are our most important weapon in the treatment of bacterial infections, including life-threatening hospital infections. Approximately 70% of all known antibiotics are produced by actinomycetes, whereat streptomycetes make up the largest part of it. Over time antibiotic resistances have become a huge major threat to public health and thus it is urgently needed to find new efficient antibiotics. The bacterial ribosome is a hot spot for the action of many successful antibiotics. Notably, not all promising binding sites at the ribosome have been therapeutically exploited. The aim of this project is to characterize new and/or underexplored protein synthesis inhibitors.
Streptomyces aureofaciens TÜ1-01, a bacterium from the Tübinger strain collection, produces high levels of α-lipomycin, an orange-colored acyclic polyene antibiotic, which acts against gram-positive bacteria, including some pathogenic species, such as *Staphylococcus aureus*.[1] *Streptomyces aureofaciens* TÜ1-01 was also listed in the strain collection as streptogramin producer. Streptogramin antibiotics are known protein synthesis inhibitors. In order to characterize aureogistin, culture conditions were defined, where no α-lipomycin is produced but bioactivity is maintained. Bioassay screens with the culture extracts of these samples revealed activity against Gram-positive bacteria, including *Bacillus subtilis* and *Staphylococcus aureus*. Detailed examinations with *in vitro* transcription/translation assays showed

that this agent has an inhibitory effect on the translation of *E. coli* and thus may be a promising protein synthesis inhibitor.

References

[1] Kunze B, Schabacher K, Zähner H and Zeeck A. (1972) Metabolic Products of Microorganisms. *Arch. Mikrobiol*. 86: 147-174.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

861/SMBP

An Antibiotic from "Microbial Dark Matter" Targets Cell Wall Biosynthesis

C. Marx*[1], P. Barac[2,3], G. König[2,3], T. Schneider[1,3]
[1]*Pharmazeutische Mikrobiologie der Universität Bonn, Bonn, Germany*
[2]*Pharmazeutische Biologie der Universität Bonn, Bonn, Germany*
[3]*Deutsches Zentrum für Infektionsforschung, Bonn, Germany*

Novel antibiotics with unprecedented mechanisms of action are urgently needed to overcome rising resistance. Analysis of the mode of action of an antibiotic and identification of the molecular target are integral components of the drug development process. Without this detailed knowledge, rational drug design is strongly hampered.
The novel antibiotic, isolated from a previously uncultured bacterium, shows potent antibacterial activity against various pathogenic bacteria, including methicillin-resistant *S. aureus* (MRSA) and vancomycin intermediate *S. aureus* (VISA).
First experiments using *B. subtilis*- and *S. aureus*-based bioreporters provide evidences that the antibiotic induces the cell wall stress stimulon (CWSS) pointing towards cell wall biosynthesis as putative target pathway. In depth, biochemical analyses identified the molecular target and mechanism of action.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

862/SMBP

Development of catabolite repression deficient mutants strains of *C. acetobutylicum* for ABE fermentation from hemicellulose material

J. Mueller*[1], C. Pires[2], N. Saunders[2], W. Liebl[1], A. Ehrenreich[1]
[1]*TU Muenchen, Dept. of Microbiology, Freising, Germany*
[2]*Brunel University London, Chair of Systems and Synthetic Biology, Uxbridge, United Kingdom*

Conventional acetone-butanol-ethanol (ABE) fermentations with *C. acetobutylicum* using feedstocks from renewable resources such as starch compete with food production. Therefore, hemicellulose containing substrates such as substrates prepared from lignocellulose with a large share of pentoses have recently come into focus. As these feedstocks always contain glucose that represses pentose utilization, the development of strains that completely degrade the pentoses in the presence of glucose is essential for efficient butanol production from such substrates.
Carbon catabolite repression (CCR) is well studied in model organisms such as *E. coli* or *B. subtilis* but unfortunately only little is known about it in *C. acetobutylicum*. Therefore, we developed a method for the isolation of mutants that lack catabolite repression. *C. acetobutylicum* wild type was cultivated in continuous cultures on a phosphate-limited minimal medium containing small amounts of glucose and excess xylose. Glucose concentration was set to limit clostridial growth in order to promote growth of mutants that can use both xylose and glucose simultaneously and finally overgrow the mutant. Also, chemical mutagenesis using ethyl methanesulfonate was done. These mutagenesis experiments were followed up by a subsequent cultivation of the cells on xylose and the sugar analogue 2-deoxy-D-glucose, which allowed the isolation of mutants with defects in catabolite repression. Those mutants showed complete degradation of a synthetic medium containing a major share of arabinose and xylose as well as glucose and galactose and significantly increased butanol production compared to the wild type. Moreover, those mutants showed an increased butanol production from hemicellulose hydrolysates containing glucose, xylose and arabinose. Genomic sequencing of the mutants revealed frameshift mutations in the glucose- and mannose-specific phosphotransferase systems (PTS) as well as in relevant permeases and the *sigD/whiG* sigma factor family. These new findings will be used to further investigate the role of sugar transport systems in catabolite repression of Clostridia by site-directed mutagenesis using the Clostron system and a Crispr/Cas-based clean deletion system that is currently developed in our lab. Next, metabolic engineering will be done in other solventogenic Clostridia such as *C. saccharobutylicum* to create new strains for improved ABE fermentations.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

863/SMBP

Antimicrobial Synthetic Cyclopeptides Activity on *Staphylococcus aureus*

H. Rudilla[1], E. Fusté[2], S. S. Eulalia[1], F. Rabanal[3], T. Vinuesa[1], M. Vinas*[1]
[1]*University of Barcelona, Pathology & Experimental Therapeutics, Hospitalet, Spain*
[2]*University of Barcelona, Public Health, Mental Health and Perinatal Nursing, Hospitalet, Spain*
[3]*University of Barcelona, Organic Chemistry, Barcelona, Spain*

Polymyxins are natural cationic antimicrobial peptides used in the clinic to treat multidrug-resistant Gram-negative infections. It is known that the interaction of polymyxins with lipid A of the outer membrane is essential for their antimicrobial action on Gram-negatives. Since Gram-positives lack outer membrane they are inherently resistant to polymyxins including polymyxin B and colistin.
A series of synthetic peptides was performed manually following standard Fmoc/tBu procedures using DIPCDI/HOBt activation on Rink amide resin. Once assembled, cleavage of the peptides from the resin was carried out by acidolysis with Trifluoroacetic acid /triisopropylsilane/water (95:3:2, v/v) for 90 min. TFA was removed with a stream of nitrogen gas. The oily residue was treated with dry diethyl ether, and the precipitated peptide isolated by centrifugation. Homogeneity of the crude peptide was assessed by analytical HPLC on Nucleosil C18 reverse-phase columns (4 mm[1] 250 mm, 5 m particle diameter, and 120 Å porous size). Elution was carried out at 1 mLmin-1 flow with mixtures of H2O containing 0.045% TFA and acetonitrile containing 0.036% TFA (UV detection at 220 nm). Cyclization of the peptide was carried out in 5% dimethylsulphoxide aqueous solution for 24 h and lyophilized twice. Products were inspired in colistin molecule (Rabanal et al., 2015)
The antimicrobial activity of five of these novel synthetic cyclopeptides against methicillin susceptible (MSSA) and methicillin resistant (MRSA) *Staphylococcus aureus* was determined by the microdilution method according to the Clinical and Laboratory Standards Institute (CLSI). Time-kill kinetics were drawn at sub-inhibitory concentrations, MIC concentrations and concentrations higher than MIC.
Values of MIC as well as time kill curves show that these novel synthetic cyclopeptides were active against both MSSA and MRSA, with MICs ranging from 2 to 32 mg/L. These open persectives in the search of alternatives for the treatment of resistant -*S. aureus* infections, despite the mechanism of action remains unknown, but the report of Zhao et al (2016).

References
[1] Rudilla H, Fusté E, Cajal Y, Rabanal F, Vinuesa T, Viñas M. Synergistic Antipseudomonal Effects of Synthetic Peptide AMP38 and Carbapenems. Molecules. 2016 Sep 12;21(9). pii: E1223. doi: 10.3390/molecules21091223.
[2] Rabanal F, Grau-Campistany A, Vila-Farrés X, Gonzalez-Linares J, Borràs M, Vila J, Manresa A, Cajal Y. A bioinspired peptide scaffold with high antibiotic activity and low in vivo toxicity. Sci Rep. 2015 May 29;5:10558. doi: 10.1038/srep10558.
[3] Zhao J, Cheah SE, Roberts KD, Nation RL, Thompson PE, Velkov T, Du Z, Johnson MD, Li J. Transcriptomic Analysis of the Activity of a Novel Polymyxin against Staphylococcus aureus. mSphere. 2016 Jul 27;1(4). pii: e00119-16. doi: 10.1128/mSphere.00119-16

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

864/SMBP
Deconvolution of luminescence cross-talk in high-throughput gene expression profiling
M. Mauri[1], S. Vecchione*[1], G. Fritz[1]
[1]*Loewe Center for Synthetic Microbiology, Marburg, Germany*

In the recent years, luciferase cassettes have become standard genetic tools to monitor gene expression in real time and in high throughput. Compared to reporter gene assays based on fluorescence proteins, luciferase assays have a superior signal-to-noise ratio, since they do not suffer from the high auto-fluorescence background of bacterial cells, and are, in principle, only limited by the sensitivity of the photo detector. However, at the same time luciferase reporters have the drawback of emitting a constant glow upon induction, which can lead to undesired cross-talk between neighbouring wells on a microplate. Indeed, we find that the scattering light from a highly luminescent well affects reads in more than 50% of the wells in a black 96-well plate, which is specifically designed to minimize such bleed-though. In order to overcome this limitation, we developed a computational method to correct for luminescence bleed-through and estimate the true luminescence activity for each well of a microplate. As the sole input to our algorithm the signals measured from a calibration plate is needed, in which the light emitted from a single luminescent well serves as an estimate of the light-spread function – similar to a point-spread function known from optical microscopy. From this light-spread function the algorithm creates a deconvolution matrix, which can be used to correct any other measurement obtained under the same technical conditions. Here, we demonstrate that our correction preserves low level signals that are close to the background and show that it is universally applicable to different kinds of microplate readers and plate types. From our algorithm, we developed a software tool that can be freely downloaded either as MATLAB code or executable file. In summary, our software provides a simple and flexible one-click solution to luminescence cross-talk in high-throughput gene expression analyses.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

865/SMBP
Marine microorganisms as degraders of cellulose may contribute to the production of renewable biofuels
N. Wiese[1], R. Schmaljohann[1], I. Sottorff[1], A. Villalobos[1], A. V. Renuse[1], J. Wiese*[1], J. F. Imhoff[1]
[1]*GEOMAR Helmholtz Center for Ocean Research Kiel, Marine Microbiology, Kiel, Germany*

Objective: There is an increasing demand for energy worldwide. Energy from renewable biomass, such as the biofuel ethanol, is produced already to a remarkable extent and it proportion is expected to increase further. Because concerns are raised against the use of edible plants such as corn or sugar cane, the use of non-food cellulosic biomass from e.g. waste wood is of special interest for the production of biofuels. Microorganisms from terrestrial origin, like the fungus *Trichoderma reesei* or the bacterium *Clostridium thermocellum*, are known to produce a set of enzymes involved in the degradation of the polymer cellulose into monomers, which can be metabolized to ethanol and other biofuels. In contrast, our knowledge about the potential of fungi and bacteria from marine habitats to produce cellulolytic enzymes is very limited. Our study aims to increase our knowledge on the ability of marine microbes to decompose cellulose.
Methods: Fungal isolates and actinobacteria from seawater, marine sediments, drift wooden, and marine macroorganisms were studied in regard to their capability to use various materials containing cellulose as sole carbon source. The strains were classified morphological and by phylogenetic analyses using sequences of ITS1-5.8S rRNA-ITS2 fragments and the 16S rRNA gene, respectively.
Results: Almost all representatives of 16 genera affiliating to diverse fungal classes as well as most of the bacteria belonging to a broad range of actinobacterial taxa were able to grow on media supplemented with cellulose. Also the production of cellulolytic exoenzymes was observed.
Conclusion: Marine microorganisms have a high potential to degrade cellulose, capabilities that can be used to treat plant and waste material containing this polymeric sugar. They may produce new cellulases exhibiting high stability and activity and thereby may enhance the possibilities of industrial production of biofuels.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

866/SMBP
Reduction of organic acids to alcohols in the thermophilic bacterium *Thermoanaerobacter* sp. strain X514
M. Kuntz*[1], F. Langschied[1], M. Basen[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt / Main, Germany*

Introduction: The reduction of organic acids to their corresponding alcohols has been studied in Clostridia (1) and in a genetically modified strain of *Pyrococcus furisous* (2). Organic acid may either be activated to their CoA-esters and then reduced by aldehyde dehydrogenase (ALDH) or directly reduced to the aldehyde by an aldehyde:ferredoxin oxidoreductase (AOR), as shown for *P. furiosus.* The genome of *Thermoanaerobacter sp*. strain X514 (*T*. X514) contains both ALDH and AOR. The strain ferments C5 and C6 sugars and to mainly ethanol and hydrogen, acetate, lactate, making it potentially very interesting for biotechnological applications.
Objectives: Our aim was to determine whether *T*. X514 reduces organic acids to alcohols in the presence of sugars as electron donors.
Material and Methods: Therefore, cell suspension experiments were performed with *T*. X514 in the presence of organic acids. AOR and ALDH activities were determined in cell-free extracts to evaluate whether the first reduction is catalyzed by AOR and

reduced ferredoxin or by a bifunctional NADH-dependent ALDH like AdhE.
Results: *T.* X514 reduced up to 20 mM of organic acids to their corresponding alcohols such as butanol, isobutanol, hexanol or phenylalcohol with a rate up to 6,3 mmol h^{-1} g^{-1}. Activities of both ALDH and AOR were present in the cell-free extracts, 0.13 U mg^{-1} and 0.12 U mg^{-1} were determined with isobutyraldehyde as substrate. Furthermore, growth experiments with addition of isobutyrate were performed to differentiate between the pathways. Interestingly, *T.* X514 grew better in the presence of isobutyrate. The specific activity of AOR increased to 0.81 U mg^{-1}, and at the same time the specific ALDH activity decreased to 0.05 U mg^{-1}. The reaction of the organism to the external acceptor implies that the first step of the reduction of organic acids may be performed by AOR.
Conclusion: Here we show that *T.* X514 reduced different organic acids to their corresponding alcohols in the presence of sugars as electron donors. Both activities of AOR and ALDH were measured and in a similar range. Therefore it could not ultimately be determined if organic acid reduction was catalyzed by AOR, or if an ALDH was involved, or both. Currently we use a combination of genetic and microbiological and biochemical methods to elucidate the pathway and enzymes involved in the production of ethanol from sugars and in the reduction of organic acids to their corresponding alcohols.

References
[1] Simon H, White H, Lebertz H, Thanos I (1987) Angew Chem Int Ed Engl 26: 785–787.
[2] Basen M, Schut GJ, Nguyen DM, Lipscomb GL, Benn RA, Prybol CJ, Vaccaro BJ, Poole FL, Kelly RM, Adams MWW (2014) Proc Natl Acad Sci USA 111:17618-17623.

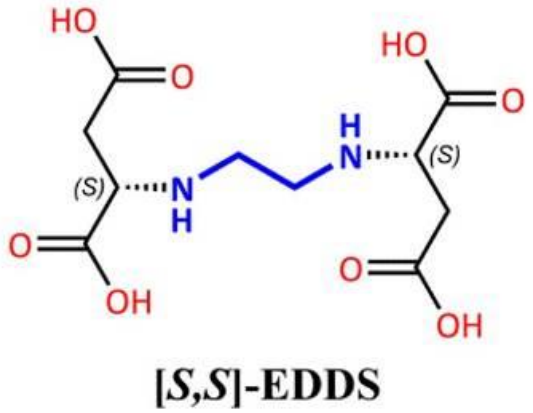
Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

867/SMBP
Ethylenediamine-disuccinate ([*S,S*]-EDDS) from *Amycolatopsis japonicum*
S. Edenhart*[1], M. Spohn[1], W. Wohlleben[1], E. Stegmann[1]
[1]*University, Microbiology/Biotechnology, Tübingen, Germany*

Chelating agents play a crucial role in the supply of microorganisms with trace elements as well as in the general ecology of microorganisms. In analogy to siderophores synthesized under iron starvation, the gram-positive soil bacterium *Amycolatopsis japonicum* produces the zincophore [*S,S*]-ethylene diamine-disuccinic acid (EDDS) under zinc limited conditions. However, already 2 µM zinc, a concentration occurring ubiquitously, inhibits the synthesis of EDDS.
EDDS is of industrial interest because it has similar properties as ethylenediamine-tetraacetate (EDTA). EDTA is widely used in industry but also as cosmetic, food, and medical additive. Due to its poor degradability, EDTA became an environmental hazard. In contrast to EDTA, EDDS (exclusively the [*S,S*]-isomer) is biodegradable. However, biotechnological production of [S,S]-EDDS was not implementable due to the complete repression of [S,S]-EDDS biosynthesis by traces of zinc.
In an alternative approach to isolate the EDDS biosynthetic gene cluster, we first aimed to understand the zinc regulation in *A. japonicum*. We identified the global zinc regulator (Zur) in *A. japonicum* by *in silico* analyses, and then proved the zinc dependent regulation of the zinc uptake system (*znuABC*) via transcriptional analysis and EMSA experiments. Following the characterization of the Zur binding motive, the *A. japonicum* genome was screened for the presence of further Zur binding motives. This enabled the identification of the putative [*S,S*]-EDDS biosynthesis genes. The deletion of selected genes resulted in mutants which were not able to produce [*S,S*]-EDDS anymore, confirming the involvement of these genes in [*S,S*]-EDDS biosynthesis [1]. In order to deactivate the zinc repression we deleted the *zur* gene. The resulting Δ*zur* mutant synthesized EDDS even in the presence of high zinc concentrations. Continuous cultivation of this mutant in an metal fermenter using a cheap mineral medium delivered a high yield of EDDS (17 g/l).

References
[1] M. Spohn, W. Wohlleben, E. Stegmann (2016). Elucidation of the zinc-dependent regulation in *Amycolatopsis japonicum* enabled the identification of the ethylenediamine-disuccinate ([*S,S*]-EDDS) genes. *Environ. Microbiol*. 18:1249-1263. doi: 10.1111/1462-2920.13159.

Figure 1



[*S,S*]-EDDS

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

868/SMBP
Development of an enzyme cascade process for the production of chiral β-amino acids
U. Engel*[1], C. Lohman[1]
[1]*Karlsruhe Institute of Technology (KIT), IBLT II, Technical Biology, Karlsruhe, Germany*

Chiral β-amino acids are valuable building blocks for pharmaceuticals and fine chemicals (Pilsl and Reiser 2011). Thus this work focuses on the development of a modified hydantoinase process using racemic dihydropyrimidines as educts for the production of chiral β-amino acids. The process is to be based on two enzymes. A cyclic amidase will be used for hydrolytic cleavage of the dihydropyrimidine ring followed by the reaction of a linear amidase able to decarbamoylate *N*-carbamoyl β-amino acids.
While a screening for enzymes catalyzing the decarbamoylation step is ongoing it was already demonstrated in previous work that hydantoinases can hydrolyze racemic 6'-substituted dihydropyrimidines to the corresponding *N*-carbamoylated β-amino acids (Engel *et al.* 2012).
To enable the utilization of these hydantoinases in an enzyme cascade process the following tasks are to be accomplished:

- - Optimization of expression conditions and enzyme purification
- - Biochemical characterization using the model substrate phenyldihydrouracil
- - Immobilization and application in a microfluidic system

References
[1] Engel, U., Syldatk, C. and Rudat, J., *Appl. Microbiol. Biotechnol.,* 94(5), (2012), 1221-1231.

[2] Pilsl, L. and Reiser, O., *Amino Acids*, (2011), 1-10.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

869/SMBP

β-amino acid production by a hydrolase/transaminase enzyme cascade: Increasing the stability of a β-transaminase by directed evolution.

O. Buß[1], K. Rabe[2], J. Rudat*[1]
[1]*Karlsruhe Institute of Technology, BLT_II: Technical Biology, Karlsruhe, Germany*
[2]*Karlsruhe Institute of Technology, IBG_I: Molecular Evolution, Eggenstein-Leopoldshafen, Germany*

Transaminases are a powerful tool for the synthesis of chiral amines and amino acids due to their wide substrate spectrum and their high enantioselectivity [1]. The potential application of these enzymes for the production of enantiopure β-amino acids was discussed earlier [2]. The problem of using instable β-keto acids as substrates can be circumvented by coupling hydrolase and transaminase activity, starting from stable β-keto esters [3]. Suitable hydrolases have been successfully screened by statistically evaluated HTS assays [4].
A remaining challenge is the suitability of the β-transaminases discovered so far for application in technical processes with respect to long-term stability. In a recent approach we succeeded in coupling purification and functional immobilization on magnetic beads to gain a ready-to-use and recyclable β-transaminase in one single step [5].
We are now stabilizing the transaminase against higher temperatures by directed evolution
- to gain higher conversion rates due to better substrate solubility and higher catalytic activity
- to achieve general improved enzyme stability with the long-term objective to further evolve the enzyme for the acceptance of β-keto esters as substrates, thus circumventing the instable β-keto acid intermediate emerging in the hydrolase/transaminase cascade reaction.
For this purpose, we use FoldX as an efficient tool for the determination of suitable mutation sites. Resulting transaminase mutants are tested for heat stability by melting curves and activity assays.

References

[1] Dold S-M, Syldatk C, Rudat (2016) Ch. 29 in: Ramesh Patel (Ed.): *Biocatalysis – Green Technology*, John Wiley & Sons ISBN: 978-1-118-82229-6 DOI: 10.1002/9781118828083.ch29
[2] Rudat J, Brucher B, Syldatk C (2012) *AMB Express* 2: 11
[3] Mathew S, Jeong S-S, Chung T, Lee S-H, Yun H (2015) *Biotechnol J* 11:1, 185
[4] Buß O, Jager S, Dold S-M, Zimmermann S, Hamacher K, Schmitz K, Rudat J (2016) *PLoS ONE* 11:1
[5] Dold S-M, Liyin C, Rudat J (2016) *Eng Life Sci* 16:6, 568.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

870/SMBP

Acetaldehyde metabolism of *Acetobacterium woodii*

D. Trifunovic*[1], N. Berghaus[1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt / Main, Germany*

Introduction: Acetogenic bacteria can utilize a broad variety of different substrates like sugars, alcohols and carboxylic acids. The well-studied acetogen *Acetobacterium woodii* can grow on different alcohols like ethanol or ethylene glycol (1, 2) and the pathways have been elucidated recently (3, 4). Acetaldehyde is an intermediate in both pathways.
Objectives: The question arose whether *A. woodii* is able to use acetaldehyde as carbon and energy source and if so, how acetaldehyde is utilized.
Material & Methods: Growth of *A. woodii* was analysed. The acetaldehyde utilization in growing and resting cells was analysed by gas chromatography. Enzymatic measurements in cell free extracts and western blot analysis revealed the enzymes involved in acetaldehyde utilization.
Results: *A. woodii* was able to grow on acetaldehyde (5 mM) and during growth on acetaldehyde, the substrate was converted to acetate and ethanol. Activities of a NADH:acetaldehyde oxidoreductase and a CoA-dependent acetaldehyde:NAD+ oxidoreductase were determined in cell-free extract. Western blot analysis revealed the presence of an alcohol dehydrogenase and of a CoA-dependent acetaldehyde dehydrogenase.
Conclusion: *A. woodii* is able to grow on small amounts of acetaldehyde. Acetaldehyde is disproportionated to acetate and ethanol by a CoA dependent acetaldehyde dehydrogenase and an alcohol dehydrogenase. In the stationary phase, the ethanol produced is converted to acetate, most likely by AdhE.

References

[1] H Buschhorn, P Dürre and G Gottschalk (1989) Production and utilization of ethanol by the homoacetogen *Acetobacterium woodii*. Appl. Environ. Microbiol. 55:1835-1840
[2] B Schink and M Stieb (1983) Fermentative degradation of polyethylene glycol by a strictly anaerobic, Gram-negative, nonsporeforming bacterium, *Pelobacter venetianus* nov. Appl. Environ. Microbiol. 45:1905-1913
[3] J Bertsch, AL Siemund, F Kremp, and V Müller (2015) A novel route for ethanol oxidation in the acetogenic bacterium *Acetobacterium woodii*: the AdhE pathway. Environ. Microbiol. 18: 2913-2922
[4] D Trifunovic, K Schuchmann, V Müller (2016) Ethylene glycol metabolism in the acetogen *Acetobacterium woodii*. Bacteriol 189**:** 1058-1065

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

871/SMBP

Construction of *G. oxydans* strains for the production of L-erythrulose and 5-ketogluconate

S. Gruber*[1], D. Kostner[1], M. Mientus[1], W. Liebl[1], A. Ehrenreich[1]
[1]*Technical University of Munich, Chair of Microbiology, Freising, Germany*

Acetic acid bacteria like *Gluconobacter oxydans* are nonpathogenic organisms that are utilized in numerous biotechnological processes. Due to the possession of membrane-bound dehydrogenases, acetic acid bacteria can incompletely oxidize a wide variety of sugars, sugar alcohols and polyols. Together with the ability of *Gluconobacter* to grow in highly concentrated substrate solutions, this can be used to efficiently produce fine and bulk chemicals, which are not readily available by organic chemistry.
This study concentrates on the rational construction of strains for the production of L-erythrulose from *meso*-erythritol and 5-ketogluconate from D-glucose. Both chemicals are products of high industrial interest. L-erythrulose is used in the cosmetic industry as a self-tanning agent. 5-ketogluconate, in contrast, is a stable compound that serves as a precursor for vitamin C production.
We have developed methods for the markerless deletion of the native membrane-bound dehydrogenases from *G. oxydans* 621H in order to avoid competing reactions by membrane bound dehydrogenases, which might have overlapping substrate spectra. In the resulting multi-deletion strain *G. oxydans* BP.9, lacking all its native membrane bound dehydrogenases, the polyol

dehydrogenases from different acetic acid bacteria were expressed in order to study the production of L-erythrulose. For the selective accumulation of 5-ketogluconate, the strain *G. oxydans* BP.9 was transformed with shuttle vectors expressing simultaneously the genes of glucose dehydrogenases and polyol dehydrogenases. The direct comparison of polyol dehydrogenases from different organisms revealed significant variations in the oxidative potential and the substrate oxidation spectra of those enzymes. Oxidation of the substrates was quantified in a whole cell 2,6-dichlorophenolindophenol (DCPIP) activity assay. Additional optimization was accomplished testing expression of the membrane bound dehydrogenases located on plasmids and the chromosome with different promoters, showing different product yields of L-erythrulose and 5-ketogluconate as measured by quantification using high-performance liquid chromatography (HPLC). Further studies should result in strains suitable for the industrial production of L-erythrulose and 5-ketogluconate.
Selective expression of membrane bound dehydrogenases of acetic acid bacteria could be a new general strategy for rational design of acetic acid bacteria strains with high oxidative activity, capable of regio- and stereo-specific oxidative fermentations that are avoiding competing and further reactions often found in wild type strains. The usage and combination of enzymes from various strains should allow development of new biotechnological processes.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

872/SMBP
Physiological barriers for glucose utilization in *Methanosarcina acetivorans*
M. Richter*[1], C. Sattler[1], R. K. Thauer[2], M. Rother[1]
[1]*Technische Universität Dresden / Institut für Mikrobiologie, Professur für Mikrobielle Diversität, Dresden, Germany*
[2]*Philipps-Universität Marburg / Max-Planck-Institut für terrestrische Mikrobiologie, Marburg, Germany*

Methanogenesis is the last step in degradation of organic matter in anoxic ecosystems and is only carried out by methanogenic archaea. The major substrates utilized as carbon and energy source by these so-called methanogens are H2 + CO2, methylated compounds and acetate. The model organism *Methanosarcina acetivorans*, one of the most versatile methanogens, is capable to use various methylated compounds, CO, acetate and also pyruvate as the sole carbon and energy source for growth. However, *M. acetivorans* also encodes most functions required for glucose oxidation, i.e., formation of methane from glucose, except for those of carbohydrate uptake (and activation). By complementing *M. acetivorans* with the gene encoding the *Zymomonas mobilis* glucose facilitator (*glf*), a strain was generated capable of converting glucose to methane. However, the rate of glycolytic methane formation in the transgenic strain was very low, consistent with the very low specific activities of the glycolytic enzymes. Furthermore, the strain was not able to grow with glucose as the sole carbon and energy source and methanol-dependent growth was even impaired in the presence of glucose.
In order to evolve the glycolytic capabilities in *M. acetivorans via* selection (e.g., by up-regulating the activities of the glycolytic enzymes), a strain was generated that lacked carbon monoxide dehydrogenase/acetyl coenzyme A synthase and contained Glf. As catabolism (methanogenesis) was separated from anabolism in this strain, providing glucose as the only anabolic supplement should force the strain to utilize it for biosynthesis. However, no methanol-dependent growth was observed, when it depended on glucose as the sole carbon source. To elucidate why the organism does not effectively use glucose, neither for catabolism nor anabolism, the fate of intracellular glucose was examined in growing cultures and in resting cells. The results obtained indicate that, while the cells effectively take up exogenous glucose, it is not oxidized further in appreciable amounts but mainly converted into other (polymeric) carbohydrates, including glycogen. The physiological consequences for *M. acetivorans* of overproducing storage compounds, and possible avenues to avoid it, will be discussed.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

873/SMBP
A *pyrE*-based genetic system for the thermophilic syngas-fermenting bacterium *Thermoanaerobacter kivui*
H. Laura*[1], G. Irina[1], M. Volker[1], B. Mirko[1]
[1]*Goethe University Frankfurt, Molecular Microbiology & Bioenergetics, Frankfurt / Main, Germany*

Introduction: Fermentations at elevated temperatures represent an interesting alternative to current procedures in biochtechnology. At high temperatures, chemical reactions proceed much faster, the risk of contamination is minimal and the cooling and distillation cost are lower. *Thermoanaerobacter kivui* is one of the very few acetogenic thermophilic bacteria with an optimal growth temperature of 65°C. It grows autotrophically on components of syngas: hydrogen plus carbon dioxide or on carbon monoxide and produces acetate as main product. Carbon dioxide is fixed through the Wood-Ljungdahl pathway, while hydrogen is oxidized via an electron-bifurcation hydrogenase and a hydrogen-depend carbon dioxide reductase.
Objectives: Our aim was to establish a genetic system allowing metabolic engineering.
Materials and Methods: We used a replicating *Thermoanaerobacterium-E. coli* shuttle vector as basis to develop a *pyrE*-based genetic system for *T.kivui*.
Results: *T. kivui* is naturally competent with transformation frequencies up to $1*10^{-2}$. The highest frequency was observed in the exponential phase, were genes putatively encoding proteins involved in DNA uptake, *comEA* and *comEC* upregulated by a factor of 2. Subsequently, we developed a plasmid for the deletion of *pyrE* through were either single or double homologous recombination. *PyrE* encodes for an important enzyme in pyrimidine biosynthesis, orotate-phosphoribosyltransferase. 5-Fluoroorotic acid (5`FOA) was used to select for the loss of *pyrE*. The Δ*pyrE* mutant exhibited an uracil-auxotrophy. The Δ*pyrE* mutant was complemented by uptake of a *pyrE*-containing plasmid. Growth experiments showed no differences between the growth rate and the final OD_{600} of the WT, the *pyrE* mutant and the complemented strain.
Conclusion: Here we show that *T. kivui* is highly naturally competent in the exponential growth phase. Furthermore, a markerless deletion of the *pyrE* gene was created, and the mutant strain was not able to grow without uracil. We are currently using *pyrE* as selective marker and uracil as selective agent to generate gene deletions in important biochemical pathways. For example, this genetic system may be used to investigate the acetogenesis in *T. kivui* or to generate modified strains to produce biochemicals or biofuels.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

874/SMBP
Dynamic process optimization in Biotechnology: Using succinate production as an example
L. Kyselova*[1], K. Bettenbrock[1]
[1]*Max-Planck-Institute, Magdeburg, Germany*

Succinate is a fine chemical used in many fields such as food industry, pharmaceuticals and cosmetics. It can be produced by petrochemical methods or by microorganisms. Different strategies are followed in order to improve the efficiency of the succinate

production by E. coli [1]. The succinate to glucose ratio can be raised by prohibiting the production of other fermentation products, by deletion of selected genes and by enhancing the metabolic fluxes towards succinate by gene overexpression. Typically, strains with enhanced succinate yield are characterized by low or zero anaerobic growth rates due to their inability to produce the competing alternative fermentations products. Hence two-phase cultivation methods are commonly applied to increase productivity. This project aims at the development of synthetic switches, which allow dynamic control of bioprocesses.
sRNA (small regulatory RNA) allow the dynamic switch off of genes. Small RNAs interact with the mRNA by direct base pairing, which leads in most cases to the destabilization of the mRNA and to the inhibition of translation. A major advantage of this approach in comparison to the chromosomal gene deletion is that sRNA genes can be regulated at any point in time, allowing dynamic process control [2].
The gene lacZ was selected as target, because its activity is easy to determine. The activity of lacZ after induction was significantly smaller, but our experiments showed unspecific effects on growth rate. Therefore by the method described it is possible to decrease gene expression, but it must be optimized for a tunable switching between on and off. For this aim the temperature controlled promoter will be tested.
Another possibility for dynamic process control is using CRISPRi system [3]. CRISPRi is a tool that allows for sequence specific repression or activation of gene expression in many organisms. CRISPRi system consists of a catalytically inactive Cas9 protein, lacking endonuclease activity but with DNA binding function. Genes can be regulated by complementary base-pairing with a single guide RNA (sgRNA) to specific DNA targets. The compex formed sterically blocks the progression of RNA polymerase and inhibits transcription. Two gene lacZ and ptsG were tested with this method resulting in greater transcriptional repression but independent from inductor. However, the data show that the system described by [3] does not allow for a dynamic control. For dynamically process control we will introduce the inducible dCas9 expression cassette into the genome. Hence, we are aiming at an optimization of this system with regard to its utility in controlling succinate production processes.

References
[1] Sánchez et al. Novel pathway engineering design of the anaerobic central metabolic pathway in Escherichia coli to increase succinate yield and productivity. Metabolic engineering 7, 229-239, 2005
[2] Na et al. Metabolic engineering of Escherichia coli using synthetic small RNAs. Nature Biotechnol. 31 (2):170-174), 2013
[3] Qi et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell 152(5): 1173-1183, 2013

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

875/SMBP
Genome-based comparison of *Moorella thermoacetica* strains and taxonomic reclassification of the species *Moorella thermoautotrophica*
A. Poehlein*[1], S. Redl[2], C. Esser[3], F. Bengelsdorf[3], T. Jensen[2], C. Jendresen[2], P. Dürre[3], A. Nielsen[2], R. Daniel[3]
[1]*Georg-August-University Goettingen, Genomic and Applied Microbiology, Goettingen, Germany*
[2]*Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Hørsholm, Denmark*
[3]*Institut für Mikrobiologie und Biotechnologie, Universität Ulm, Ulm, Germany*

The interest from the research community and industry in acetogenic bacteria has grown within recent years due to their potential to produce valuable compounds from syngas streams [1]. Thermophilic acetogens are of significance, as their use could reduce gas cooling requirements, allow cost-efficient recovery of products with relatively low boiling point [2] and decrease the risk of contamination. Thus, they provide potential advantages for the fermentation of syngas and production of volatile compounds. The thermophiles *Moorella thermoacetica* and *M. thermoautotrophica* are model organisms for acetogenic metabolism. Several strains originating from the cultures isolated by Fontaine et al. [EMO1] [3] are deposited in strain collections. Mainly the type strain DSM 521T and the strain ATCC 39073 served to elucidate the primary metabolism of *M. thermoacetica*. The genome of the non-type strain ATCC 39073 was sequenced in 2008 [4] and the genome sequence of the type strain DSM 521T followed last year [5]. In order to expand the knowledge of the genus, we sequenced the genomes of eleven different *M. thermoacetica* and *M. thermoautotrophica* strains. Whole genome sequencing was done using the Illumina technique. *De novo* assemblies were performed with the SPAdes software.
Sequencing revealed that *M. thermoautotrophica* DSM 1974T consists of at least two different strains. Phylogenetic analysis showed a close relationship between all the sequenced genomes, suggesting that the species of *M. thermoautotrophica* should be reclassified as *M. thermoacetica*. Despite genetic similarities, differences in genomic features were observed between the strains. Differences in compounds that can serve as carbon and energy sources for selected strains were identified. The present study contributes to increase the knowledge on the genetic diversity of *M. thermoacetica* strains, as well as some of the phenotypic traits of this important group of microorganisms.

References
[1] H. Latif, A. A. Zeidan, A. T. Nielsen, and K. Zengler, "Trash to treasure: production of biofuels and commodity chemicals via syngas fermenting microorganisms," Curr Opin Biotech, vol. 27, pp. 79–87, 2014.
[2] A. M. Henstra, J. Sipma, A. Rinzema, and A. J. M. Stams, "Microbiology of synthesis gas fermentation for biofuel production," Curr Opin Biotech, vol. 18, no. 3, pp. 200–206, 2007.
[3] F. E. Fontaine, W. H. Peterson, E. McCoy, M. J. Johnson, and G. J. Ritter, "A new type of glucose fermentation by Clostridium thermoaceticum," J Bacteriol, vol. 43, no. 6, pp. 701–715, 1942.
[4] E. Pierce et al, "The complete genome sequence of Moorella thermoacetica (f. Clostridium thermoaceticum)," Environmental microbiology, vol. 10, no. 10, pp. 2550–2573, 2008.
[5] A. Poehlein et al, "Complete genome sequence of the type strain of the acetogenic bacterium Moorella thermoacetica DSM 521T," Genome announcements, vol. 3, no. 5, pp. e01159-15, 2015.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

876/SMBP

Microbial proteins for the generation of 3D cell culture templates

N. Bodenberger*[1], D. Kubiczek[1], A. Wittgens[1], F. Rosenau[1]
[1]*Ulm University, Center for Peptide Pharmeceuticals, Ulm, Germany*

Hydrogels have gained considerable attention in recent years due to their unique properties with an increasing numbers of publications each year. Hydrogels are insoluble three dimensional networks that can maintain huge amounts of water while being insoluble. They are applied in different fields ranging from construction industries to biological, medical and biotechnological applications. One major goal of many groups worldwide is the use of hydrogel systems as a growth substrate for 3D cell cultures. As the matrix can bind huge amounts of water, the network can mimic the extracellular matrix to a certain extend. Furthermore, the hydrogel properties like swelling ratio, diffusion, elasticity, pH and temperature resistance, rigidity, degradation pattern and biocompatibility can be adjusted according to the intended use. Protein based systems as a subclass of modern hydrogels have tremendous potential due to the adjustability, the exact definition of the protein network and the huge amount of accessible target sites within the material. However, protein hydrogels are often way too expensive to be used on a regular basis for many applications.
In this study, we aimed on producing and establishing a protein hydrogel from a whole cell protein extract. As a reliable, cheap and well-characterized source for this, *Saccharomyces cerevisae* protein was used to form three dimensional networks with adjustable mechanical properties.
Essential features of hydrogels for cell culture are the pores within the 3D matrix: they have to be big enough to guarantee a proper and fast removal of toxic cellular intermediates while nutrition and oxygen have to reach cells immediately. To manipulate pore size, a freeze-drying approach was used and the resulting pores were analyzed with confocal laser scanning microscopy, revealing adjustable 3D architectures. Another crucial feature for the use of hydrogels in 3D cell cultures is the reversibility of the system. By increasing the pore sizes within the material, the enzymatic degradation of the network could be accelerated and a complete elimination of the network was accomplished after 80 – 200h, depending on the hydrogels composition. Furthermore, the adjustability of the elastic properties of this protein based system was investigated using atomic force microscopy. Finally, the feasibility of the system for cell culture applications was shown by incorporating a cell adhesive RGD moiety into the network and demonstrating the growth of human breast cancer cells (MCF7) and adenocarcinomic human alveolar basal epithelial cells (A549) on the hydrogels surface. Those macroporous hydrogel which are produced with proteins of one of the most well-characterized microorganisms, *Saccharomyces cerevisae*, fulfilled all requirements of a hydrogel template for the cultivation of mammalian cells under 3D conditions.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

877/SMBP

Fundamental research of PHB metabolism in cyanobacteria for an increased yield of carbon-neutral bioplastics.

M. Koch*[1], W. Hauf[1], K. Forchhammer[1]
[1]*University Tuebingen, Oragnismic Interactions, Tübingen, Germany*

Under nutrient starvation, certain cyanobacteria accumulate several storage polymers like Polyhydroxybutyrate (PHB) and glycogen (Klotz *et al.*, 2016). PHB is of high biotechnological relevance since it can be used as a source for bio-degradable plastics. Recently various experiments have been conducted to elucidate how the PHB metabolism functions in the model strain *Synechocystis* sp. PCC 6803. We could identify the first cyanobacterial Phasin (PHB coating protein) PhaP and demonstrate that PHB granules contain no phospholipids (Hauf *et al.*, 2015)(Bresan *et al.*, 2016).
Still, fundamental questions about the physiological function and metabolism of PHB remain unanswered. According to text book knowledge, PHB could serve as a storage polymer, supplying carbon and energy during starvation conditions. However, we could not detect any phenotypic difference between WT and PHB-free cells under conditions of PHB formation such as nutrient starvation conditions (Klotz *et al.*, 2016). Hence, several growing conditions will be tested in order to find a situation where the presence of PHB is advantageous for the cells. This could provide an efficient screening method by directed evolution for mutants showing a PHB overproducing phenotype.
In a second approach, different metabolic engineering strategies will be applied to create *Synechocystis* strains with increased PHB content. One aspect could be to alter the intracellular pool of the PHB precursor, Acetyl-CoA. By engineering the central regulator protein PII, we were able to demonstrate interactions with the acetyl-CoA carboxylase leading to altered Acetyl-CoA levels (Hauf *et al.*, 2016). By increasing the metabolic flow towards Acetyl-CoA, such as by modifying the PII signaling system or introducing additional pathways, PHB synthesis could be further increased. The success of this approach will be validated via C-flux-monitoring and the most promising interventions combined.
Bibliography

References

[1] Bresan, S., A. Sznajder, W. Hauf, K. Forchhammer, D. Pfeiffer & D. Jendrossek, (2016) Polyhydroxyalkanoate (PHA) Granules Have no Phospholipids. *Scientific reports* 6: 26612.
[2] Hauf, W., K. Schmid, E.C. Gerhardt, L.F. Huergo & K. Forchhammer, (2016) Interaction of the Nitrogen Regulatory Protein GlnB (PII) with Biotin Carboxyl Carrier Protein (BCCP) Controls Acetyl-CoA Levels in the Cyanobacterium Synechocystis sp. PCC 6803. *Frontiers in microbiology* 7: 1700.
[3] Hauf, W., B. Watzer, N. Roos, A. Klotz & K. Forchhammer, (2015) Photoautotrophic Polyhydroxybutyrate Granule Formation Is Regulated by Cyanobacterial Phasin PhaP in Synechocystis sp. Strain PCC 6803. *Applied and environmental microbiology* 81: 4411-4422.
[4] Klotz, A., J. Georg, L. Bučinská, S. Watanabe, V. Reimann, W. Januszewski, R. Sobotka, D. Jendrossek, W. Hess & K. Forchhammer, (2016) Awakening of a dormant cyanobacterium: Resuscitation of chlorotic cells reveals a genetically determined program. *Current biology : CB* In Press.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

878/SMBP

A microbial photosensitizer protein as a potential anticancer drug

N. Bodenberger*[1], D. Kubiczek*[1], S. Nour El Din[1], F. Rosenau[1]
[1]*Ulm University, Center for Peptide Pharmeceuticals, Ulm, Germany*

The research on a wide range of microorganisms offers the potential to discover and evaluate novel bioactive compounds. The cupredoxin azurin from *Pseudomonas aeruginosa* which is part of its respiratory machinery has been shown to also exhibit a cancer cell specific cell penetration activity as well as an antiproliferative effect. Those properties could be traced back to a short, 28 amino acid peptide sequence, designated as P28. By fusion of this cell penetrating peptide with a light oxygen voltage domain (LOV), which was derived from the bacterial blue light photoreceptor YtvA from *Bacillus subtilis*, we generate a cell penetrating photosensitizer as a potential anticancer drug. Upon excitation with blue light the production of reactive oxygen

species of the LOV domain is triggered, which further increased the toxicity against cancer cells.
In this study, the fusion protein was produced recombinantly in *Escherichia coli*. The overall aim of this study was to evaluate the potential anticancer activity of this fusion protein. The uptake of the construct into a model cell line (A549) was shown using confocal laser scanning microscopy and electrophoretic gel analysis while the effects of the designed protein on a cellular level was investigated with flow cytometry.
The fusion with the cell penetration P28 did not alter the properties of the LOV domain. The uptake of the LOV domain into A549 cells was shown to be an effect of the P28 peptide and could be detected in high amounts in the cells after several hours. Upon excitation with blue light, the amount of reactive oxygen species increased massively within the cells. Cells which were treated with this fusion product showed a cell death of about 20%. In combination with a blue light treatment of the cells, the cytotoxicity of the fusion protein could be increased up to 90%. The P28 peptide is currently in clinical phase I and shows promising results. As the LOV domain forms radicals in the absence of oxygen, we see a potential application even for the treatment of anoxic cancer tumors microenvironments. Another advantage is the specificity towards cancer cells while other cells are not affected by the reactive oxygen formation of the product, which might result in reduced side effects.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

879/SMBP
Optimizing the 3-Hyxdroxypropionate Photorespiratory Bypass for Direct and Efficient CO_2 Fixation
P. Pfister*[1], J. Zarzycki[1], T. Erb[1]
[1]*Max Planck Institute for Terrestrial Microbiology, Biochemistry and Synthetic Biology of Microbial Metabolism, Marburg, Germany*

RubisCOs inability to discriminate well enough between CO_2 and O_2 as substrates results in photorespiration. When O_2 is used instead of CO_2 toxic 2-phosphoglycolate is formed, which has to be removed and recycled into a central carbon metabolism intermediate. The recycling of phosphoglycolate leads to the release of CO_2 and ammonia. It has been estimated that in C_3-plants up to 25% of the carbon is released due to this process. To scavenge phosphoglycolate as well as refix the lost carbon additional input of ATP and reducing equivalents is required. Previously engineered photorespiratory bypasses were only able to circumvent the loss of nitrogen, but still resulted in (even higher) CO_2 release [1]. In contrast, our approach aims at direct CO_2 fixation via an additional carboxylase reaction. We proposed a cyclic bypass that is based on the 3-Hydroxypropionate Bi-Cycle, an alternative CO_2 fixation pathway found in the filamentous anoxygenic phototroph *Chloroflexus aurantiacus* [2]. In a first groundbreaking study all heterologous genes required for the bypass were successfully introduced into the genome of the model organism *Synechococcus elongatus* PCC7942, and the corresponding enzymes were shown to be active in cell extracts [3]. We have identified potential bottle necks in the activities of two enzymes. To alleviate these bottle necks we employ isoenzymes and engineered mutant enzymes with higher specific activities as well as improved substrate specificities. Newly developed time resolved high resolution mass spectroscopy methods enable us to analyze, characterize, and improve enzymatic activities in order to optimize the synthetic pathway *in vitro* before reimplementation of the bypass into *Synechococcus*.

References
[1] Erb T.J., Zarzycki J. 2016. Curr Opin Chem Biol 34:72-79
[2] Zarzycki J., Brecht V., Müller, M., Fuchs G. 2009. Proc Natl Acad Sci USA 106:21317-22.
[3] Shih P.M., Zarzycki J., Niyogi K.K., Kerfeld C.A. 2014. J Biol Chem 289:9493-500

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

880/SMBP
Convertion of biomass from waste to platform chemicals
A. Schmidt*[1], T. Klessing*[1], J. Gescher[1]
[1]*KIT, Karlsruhe, Germany*

These days, the recycling and reduction of waste is a major international topic. Using mixed biomass waste streams as a substrate for the sustainable production of platform chemicals is a major aim in bioeconomy. Therefore, we developed a workflow of fermentations to convert mixed biomass streams into single substrates for biotechnology. The series of reactions starts with a biomass fermentation under slightly acidic conditions to inhibit methane production by methanogenic organisms. Either vegetable waste or cellulose was used as substrate for this fermentation. The resulting fermentates consisted mainly out of butyrate, propionate and acetate. This fermentate was used as substrate in the anode chamber of a microbial electrolysis cell. Using an appropriate anode potential and community, it was possible to couple the elimination of butyrate and acetate to CO2 with the production of an electrical current. The elimination of total organic carbon proceeded with a rate of maximum 1.9 g total organic carbon per square metre anode surface and hour. The produced current is used on the cathode site to produce hydrogen, which can be the substrate for several further biotechnological processes, including the production of pure methane via methanogenic microorganisms. The remaining propionate containing fermentate was purified via cross flow filtration. We could show that the purified solution is a suitable medium for several organisms. As a proof of concept, *Shewanella oneidensis* and *Escherichia coli* strains were genetically engineered to generate different platform chemicals, including acetoin and butanediol, from the propionate in the purified fermentate.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

881/SMBP
Next generation rhamnolipids from renewable ressources
D. S. Rhee*[1], F. Weggenmann[2], M. Henkel[2], R. Hausmann[2], A. Wittgens[1], F. Rosenau[1]
[1]*Center for Peptide Pharmaceuticals, Ulm University, Ulm, Germany*
[2]*Institute of Food Science and Biotechnology, University of Hohenheim, Department of Bioprocess Engineering (150k), Stuttgart, Germany*

In these times of ever scarcer resources, sustainability and economy are essential components of our projects. With a total worldwide production of over 15 million tons per year, surfactants are used in many industrial applications. The available surfactants are mainly oil-based and they are only partially or slowly biodegradable. Therefore they are contributing to environmental pollution and exploitation of non-renewable petroleum reserves.
Rhamnolipids are potent biosurfactants and have been investigated as potential replacements for synthetic surfactants. Due to their environmental-friendly properties they are increasingly gaining significance in industrial applications. In addition they offer antimicrobial and antimyotic activity. Regarding to the increasing amount of pathogens resistant to antibiotics, rhamnolipids may become more popular as drugs of last resort in the future. But the economics of production is a major bottleneck in the outburst of commercialization of rhamnolipids and other biosurfactants. Expensive feedstocks and downstream-processing regarding to the applications in food-processing, cosmetics and the pharmaceutical sector account 70-80 % of the entire production costs.

Here, lignocellulosic biomass offers a profitable option. It is the most abundant renewable biological resource on earth, with a yearly production of ca. 200 billion tons and lignocellulosic feedstock is far less costly than other feedstocks like crude oil, natural gas, corn kernels and vegetable oil.
Taking into account the objective of a new method in producing biosurfactants in regard of economic and sustainability, a rhamnolipid producing S1-strain degrading lignocellulosic sugar as carbon source was yielded.
Pseudomonas putida KT2440 was chosen as host for the heterologous synthesis of rhamnolipids with the genes *rhl*A (acyltransferase) and *rhl*B (rhamnosyltransferase) from *Pseudomonas aeruginosa* to avoid the complex regulatory system and human-pathogenicity of the wild type rhamnolipid producer. In addition the genes *xyl*A (xylose isomerase) and *xyl*B (xylulose kinase) from *E.coli* DH5α were transferred for metabolizing the lignocellulosic pentose xylose. In long-term cultivations the growth of the metabolic engineered strain *Pseudomonas putida* KT2440_*rhl*AB_*xyl*AB and the amount of rhamnolipids were investigated xylose as sole carbon source in comparison to glucose.
We achieved a recombinant non-pathogenic *P. putida* strain, which synthesize rhamnolipids using xylose as exceptional carbon source in comparable amounts to the usage of glucose. With this approach we consider to establish a promissing method to synthesize rhamnolipids from renewable ressources.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

882/SMBP
Novel synthetic pathways for the assimilation of formate
S. Burgener*[1], A. Bar-Even[2], T. J. Erb[1]
[1]*MPI for Terrestrial Microbiology, Marburg, Germany*
[2]*MPI of Molecular Plant Physiology, Potsdam-Golm, Germany*

Question: The conversion of CO2 into value-added multicarbon compounds is a challenging task and currently only biological catalysts are able to perform this conversion at a reasonable rate. However, the naturally occurring CO2-fixing pathways are not operating at maximum efficiency. Synthetic pathways have been proposed to fix CO2 more efficiently and we recently demonstrated this in vitro with one such pathway (1). However, an analysis of several different carbon fixation routes revealed that formate assimilation is considerably more efficient, energetically and kinetically, than fixation of CO2 (2). Similar to CO2 assimilation, the assimilation of formate requires its activation and condensation into higher carbon compounds. Natural formate assimilation pathways are not optimal for biotechnological applications, thus the design of synthetic pathways is required.
Methods: Enzyme engineering by rational design was used to create mutant enzymes with increased specificity to the desired substrates in the pathway. The enzymes were then expressed, purified and kinetically characterized. To this end, spectrophotometric and HPLC-MS-based assays were developed and applied.
Results: The key step in the formate assimilation is the activation of formate and its subsequent conversion into multicarbon compounds. Here we present a novel route that is based on engineered variants of a formyl-CoA synthetase and a pyruvate-formate lyase.
Conclusion: Synthetic biology tools and metabolic engineering can be used to develop novel pathways for the assimilation of formate.

References
[1] Schwander et al., Science 2016
[2] Bar-Even et al., PNAS 2010

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

883/SMBP
Impact of process parameters and impurities on syngas fermentation with *Clostridium ljungdahlii* and production of malic acid from syngas.
F. Oswald[1], M. Zwick[1], C. Syldatk[1], A. Neumann*[1]
[1]*KIT, Technical Biology, Karlsruhe, Germany*

The bioliq® pilot plant at the KIT covers the complete process chain required for producing customized fuels from dry lignocellulosic biomass. The intermediate product, a tar-free, low-methane raw synthesis gas (syngas) is then used for further fuel synthesis. Since chemical synthesis has high requirements for gas purity and C/H ratio extensive conditioning of the crude syngas is necessary. Therefore the pilot plant is equipped with an innovative hot-gas cleaning system to remove impurities like HCl, H_2S, COS, CS_2, NH_3, and HCN [Dahmen et al. 2008].
Acetogenic bacteria are able to grow on syngas as sole carbon and energy source under anaerobic conditions. They convert CO/CO_2 and H_2 via Acetyl-CoA to mostly acetate and ethanol using the reductive acetyl-CoA pathway. In contrast to catalysts used in chemical synthesis these bacteria can process a broad range of CO/CO_2 to H_2 ratios and tolerate impurities like sulphur or nitrogen compounds [Griffin et al. 2012].
One major challenge of this so called syngas fermentation is the poor solubility of CO and H_2 in the fermentation broth. To overcome this limitation one could increase the *kla*-value for better mass transfer into the broth or increase the pressure in the bioreactor to obtain better solubility of the gases.
To address the above named strategies a setup of multiple 2 L bioreactors with product analysis and online gas measurement was developed in our lab. With this setup it was possible to investigate *kla*-values and substrate usage of different stirrer setups and aeration modes. This system was also used for investigating the influence of cyanide, one of the main impurities of crude syngas, during growth and product formation of *Clostridium ljungdahlii* on syngas.
Suffering from energetic limitations, yields of C4-molecules produced by syngas fermentation are quite low compared with ABE fermentation using sugars as a substrate. Fungal production of malic acid on the other hand has high yields of product per gram metabolized substrate but is currently limited to sugar containing substrates. It was possible to show malic acid production by coupling acetogenic syngas fermentation producing acetate from syngas to *Aspergillus oryzae* fermentation, using acetate as sole carbon source. The overall conversion of CO and H_2 into malic acid was calculated to 0.22 gram malic acid per gram of syngas [Oswald et al. 2016].

References
[1] Dahmen, N. et al. 2008. The Karlsruhe Proccess Bioliq®. Synthetic Fuels from the Biomass. In: R. Wengenmayr and T. Bührke (eds.) *Renewable Energy*. Weinheim: Wiley-VCH. 61-65.
[2] Griffin, D. W. and Schultz, M. A. 2012. Fuel and chemical products from biomass syngas: A comparison of gas fermentation to thermochemical conversion routes. *Envrion Prog Sustain Energy*. 31:219-224
[3] Oswald, F. et al. (2016) Sequential mixed cultures: From syngas to malic acid. *Front Microbiol* 7:1–12. doi: 10.3389/fmicb.2016.00891

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

884/SMBP
Isolation and characterisation of *Clostridium pasteurianum* strains from different habitats
D. Horne*[1], R. Daniel[1], A. Poehlein[1]
[1]*Georg-August-Universität Göttingen, Genomic and Applied Microbiology, Göttingen, Germany*

Solvents such as acetone, butanol and ethanol (ABE) are important for the chemical industry as they are used as intermediates for paints, plastics and polymers. Butanol has recently also gained attention as biofuel. Butanol can either be produced petrochemical or through fermentation, mostly performed by clostridial strains. Clostrida can ferment various organic compounds such as sugars or cellulose-based substrates. Solvent production still suffers from high substrate costs, low yields due to the toxic properties of the solvents especially butanol. One solution to lower substrate costs is the utilization of glycerol as substrate. It is produced in high amounts during biodiesel production lowering its market price. One promising candidate is the solvent producer *C. pasteurianum*. The other challenges, such as the low yield and toxicity that are also faced could be encountered by novel strains. They might exhibit a naturally occurring high butanol tolerance or otherwise show useful features. The strains could then be used to for genetical and metabolic engineering of established industrial strains or they can be used to establish novel strains.
From different habitats, including cold mud volcanos in Italy and microbial mats from hypersaline lakes of the Kritimati Atoll (Central Pacific), novel strains were isolated. The whole genomes were sequenced with an Illumina MiSeq instrument. De novo assemblies were performed with SPAdes software. The quality of the assemblies was checked using QualiMap.The metabolic capabilities of the strains were analysed with the anaerobic Biolog-ID system.
The genomes of the isolates derived from this study as well as the publically available *C. pasteurianum* genomes: DSM525 [1], ATCC6013 [2], CP1 [3], NRRL B-598 [4] and BC1 [3] were compared. The genomes varied from 3.9 Mb to 4.5 Mb. The number of genes encoded ranged from 3,700 up to 4,250. The different strains showed different substrate spectra. BioLog and genome comparisons revealed at least two wrongly annotated *C. pasteurianum* strains[EMO1] . In addition, habitat-specific differences could be identified.

References
[1] Poehlein, A. et al., 2015. Complete Genome Sequence of the Nitrogen-Fixing and Solvent-Producing Clostridium pasteurianum DSM 525.
[2] Rotta, C. et al., 2015. Closed Genome Sequence of Clostridium pasteurianum ATCC 6013
[3] http://www.ncbi.nlm.nih.gov/GenBank/
[4] Sedlar, K. et al., 2015. Complete genome sequence of Clostridium pasteurianum NRRL B-598, a non-type strain producing butanol.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

885/SMBP
Activation of Silent Gene Clusters From New Unique Actinomycetes
J. Krause*[1], W. Wohlleben[1], Y. Mast[1]
[1]*Universität Tübingen, Tübingen, Germany*

Rare habitats harbour a huge number of new actinomycetical species, which serve as a rich source for new antibiotics.1,2 Especially Indonesia, as a "biodiversity hotspot" 3, may harbour unknown novel antibiotic compounds producing species. Our project aims to identify new antibiotics from Indonesian actinomycetes.
Though actinomycetes can contain plenty of antibiotic gene clusters, most of them are silent under laboratory conditions and therefore require activation. Four strains from the Tübinger strain collection, originally isolated from Java, Indonesia, serve as initial test organisms in order to establish methods to activate silent gene cluster expression. We focus on two regulatory-based strategies:

- SARP (*Streptomyces* Antibiotic Regulatory Protein)-type regulators are activators of antibiotic biosynthesis, which bind to conserved consensus sequences at the promotor region of their target genes. A typical representative for SARP-regulators is PapR2.4 Overexpression of *papR2* in *S. lividans* leads to the activation of the silent undecylprodigiosin biosynthetic gene cluster. This makes PapR2 a promising candidate to activate expression of silent gene clusters in actinomycetes in general. PCR analysis of the genomic DNA from the Javanese strains and SARP-specific primers revealed that two of the strains contain SARP-genes. In order to activate gene cluster expression the *papR2* overexpression construct pGM190/papR2 was cloned into these strains. The transformants are currently tested on their ability to produce new bioactive compounds via zone of inhibition tests and HPLC-analysis.
- γ-butyrolactones (GBLs) are general inducers of antibiotic biosynthesis in actinomycetes. GBLs bind to specific cytoplasmic receptor proteins. By causing conformational changes a downstream signalling cascade of the receptors is initiated, which results in the induction of antibiotic biosynthesis.5 HPLC analysis revealed that supplying cultures of the Javanese strains with GBL activates the production of several known and putatively also of some unknown compounds, which will be analysed further.

References
[1] Takahashi, Y. & Omura, S. Isolation of new actinomycete strains for the screening of new bioactive compounds. *J. Gen. Appl. Microbiol.* 49**,** 141–154 (2003).
[2] Lazzarini, A., Cavaletti, L., Toppo, G. & Marinelli, F. Rare genera of actinomycetes as potential producers of new antibiotics. *Antonie Van Leeuwenhoek* 78, 399–405 (2000).
[3] Myers, N. Threatened Biotas: "Hot Spots" in Tropical Forests. *Environmentalist* 8, 187–208 (1988).
[4] Mast, Y., Guezguez, J., Handel, F. & Schinko, E. A Complex Signaling Cascade Governs Pristinamycin Biosynthesis in Streptomyces pristinaespiralis. *Appl. Environ. Microbiol.* 81, 6621–36 (2015).
[5] Bibb, M. J. Regulation of secondary metabolism in streptomycetes. *Curr. Opin. Microbiol.* 8, 208–215 (2005).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

886/SMBP
Establishment of an inducible promoter system for the obligate anaerobic *Acetobacterium woodii*
M. H. Beck*[1], F. R. Bengelsdorf[1], B. Schiel-Bengelsdorf[1], P. Dürre[1]
[1]*Universität Ulm, Institut für Mikrobiologie und Biotechnologie, Ulm, Germany*

Introduction: As one of the most studied acetogens, *Acetobacterium woodii* represents a promising candidate for the biotechnological production of bulk chemicals from CO_2. Especially interesting is the biotechnological production of high-value platform chemicals. This can be achieved via the introduction and expression of biosynthesis genes in *A. woodii* and thus shifting its metabolic pathway towards the production of the desired compound. However, pathway engineering sometimes

faces major problems since the constitutive expression of some genes appears to be detrimental to the organism.
Objective: This work was intended to identify an inducible promoter system suitable for *A. woodii* allowing the expression of genes which are normally crucial to be expressed constitutively. This system should be used to mediate the expression of any desired gene at any wanted growth stage of *A. woodii*. In addition, the respective system should exhibit a proportionality of the gene expression level in accordance with the applied inducer dose.
Methods: Several constitutive and inducible promoter systems were cloned into a Gram-positive/Gram-negative shuttle vector controlling the *gusA* gene (encoding the β-glucuronidase from *Escherichia coli*). After transformation of the constructed plasmids in *A. woodii*, the respective promoter systems were analyzed using *A. woodii* cells grown on fructose via recording the specific β-glucuronidase activity of the cell lysates. The transformation rate of MUG (4-methylumbelliferyl- β -D-glucuronide) to the fluorescent 4-MU (4-methylumbelliferone) was recorded in 96 well plates.
Results: *A. woodii* exhibited a moderate tolerance of anhydrotetracycline, thus a concentration of 200 ng/ml did not impede growth when added during the exponential growth phase and thereby allowing the usage of a tetracycline inducible promoter system. After induction, GusA assays revealed the activity of this promoter system in a concentration-dependent manner. Only evanescently low activities could be detected when cells were not induced, which is in accordance with a relatively tight regulation of this promoter system.
Conclusion: The tetracycline inducible promoter system was shown to be a feasible system for *A. woodii* and thus a very promising system for the controlled expression of genes normally causing problems when constitutively expressed.
Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

887/SMBP
Organic acid production from renewable resources by *Aspergillus oryzae*
S. Dörsam[1], C. Syldatk[1], K. Ochsenreither*[1]
[1]*Karlsruhe Institute of Technology, Technical Biology, Karlsruhe, Germany*

Introduction / Question: L-malic acid and fumaric acid are C4 dicarboxylic organic acids and considered as promising chemical building blocks. They can be applied as food preservatives and acidulants, in rust removal and as polymerization starter units. Moulds of the genus *Aspergillus* are able to produce malic acid in large quantities from glucose and other carbon sources. Despite the great potential of filamentous fungi to produce organic acids, a commercial bioprocess does not exist to date due to a lack of economic viability. Therefore, fumaric and malic acid are still exclusively derived from petroleum. To solve this problem several aspects have to be taken into account. Production costs can be reduced by using waste or low-cost substrates. A second important point is the application of renewable carbon sources in order to prevent the food or fuel-discussion which would be a problem when using glucose as carbon source. Therefore, several renewable resources were inverstigated for their suitability in organic acid production.
Methods: The malic acid producer Aspergillus oryzae is cultivated with different renewable resources in shaking flasks and bioreactors. Product concentration, by-products, yields and consumption rates will be analyzed and discussed.
Results: *Aspergillus oryzae* is able to convert a wide range of different sugars as well as sugar hydrolysates from beech wood and syngas to malic acid. It was shown that complex sugar hydrolysates don´t have negative effects on malic acid production when compared to pure sugars. Syngas can be converted to malic acid by a combined fermentation approach by coupling anaerobic with aerobic fermentation resulting in very high yields.
Conclusions: Organic acid production from lignocellulosic resources using *Aspergillus oryzae* may have the potential to be real alternative to the crude oil based process.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

888/SMBP
A comprehensive view into the transcriptional landscape of *Gluconobacter oxydans* 621H
A. Kranz*[1], T. Busche[2], J. Kalinowski[2], M. Bott[3], T. Polen[1]
[1]*Research Center Jülich GmbH, Regulatory Switches and Synthetic Biology, Jülich, Germany*
[2]*Bielefeld University, Microbial Genomics and Biotechnology, Center for Biotechnology (CeBiTec), Bielefeld, Germany*
[3]*Research Center Jülich GmbH, IBG-1, Jülich, Germany*

Question: The strictly aerobic α-proteobacterium *Gluconobacter oxydans* is used for a broad range of industrial applications due to its exceptional ability to oxidize a great variety of carbohydrates in the periplasm and release of resulting products into the cultivation medium. A consequence of this unorthodox catabolism is a low biomass yield, as only a minor fraction of the carbon and energy source is metabolized in the cytoplasm. Moreover, several enzymes required for glycolysis and tricarboxylic acid cycle are absent. The aim of our study is a comprehensive characterization of transcriptional landscapes of *G. oxydans* in order to get deeper insights in the physiology and to identify important features for transcription and translation.
Methods: For whole and primary transcriptome analysis, *G. oxydans* cells were grown in complex medium containing mannitol as carbon source under standard and several stress conditions. Total RNA was isolated and depleted of rRNA. Whole transcriptome libraries were generated using a library preparation kit from Illumina. To determine transcriptional start sites (TSSs), we prepared libraries enriched for primary 5´-transcript ends by digestion of processed transcripts [1]. In order to distinguish between actual and false positive TSSs, a known sequence was ligated to 5´-ends of non-primary transcripts, which remained in the libraries due to inefficient digestion. All libraries were sequenced with a MiSeq instrument (Illumina). Data analysis was performed using CLC Genomics Workbench and ReadXplorer [2].
Results: We were able to detect at least 700 TSSs with a drastically reduced number of false positives (ca. 50%). The TSSs allowed correction of translational starts of more than 100 coding sequences and led to an extended analysis of 5´-UTRs (untranslated regions) of protein-coding genes. Less than 10% of the analyzed transcripts are leaderless, whereas nearly 50% have a 5´-UTR length >100 nt. Further characterization of these regions revealed the existence of some riboswitches. Analysis of gene expression using the whole transcriptome libraries and screening of sequences upstream of TSSs led to the identification of promoter motifs recognized by sigma factors responsible for regulation of housekeeping genes and genes involved in stress responses. Additionally, strand-specificity of the libraries enabled the detection of more than 300 antisense transcripts, which may play a role in regulation.
Conclusions: Comprehensive characterization and definition of primary transcriptomes and whole transcriptomes provided a wealth of information including regulatory genetic elements (e.g. promoter regions, RBSs). This knowledge will be used for further analysis of regulatory mechanisms in *G. oxydans* and strain development by metabolic engineering.

References
[1] Pfeifer-Sancar et al. (2013). BMC Genomics 14: 888.
[2] Hilker et al. (2014). Bioinformatics 30. 2247-2254.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

889/SMBP

Pyruvate Formate-Lyase Enables Efficient Growth of Escherichia coli on Acetate and Formate.

S. Lindner*[1], A. Bar-Even[1], C. Cotton[1]
[1]*MPI molecular plant physiology, Potsdam, Germany*

Pyruvate formate-lyase (PFL) is a ubiquitous enzyme that supports increased ATP yield during sugar fermentation. While the PFL reaction is known to be reversible *in vitro*, the ability of PFL to support microbial growth by condensing acetyl-CoA and formate *in vivo* has never been directly tested. We employed *Escherichia coli* mutant strains that cannot assimilate acetate via the glyoxylate shunt and used carbon labeling experiments to unequivocally demonstrate PFL-dependent co-assimilation of acetate and formate. Moreover, PFL-dependent growth is faster than growth on acetate using the glyoxylate shunt. Hence, growth via the reverse activity of PFL provides a novel way for assimilation of C2 compounds into central metabolism. Such growth could further pave the way for the establishment of synthetic formate assimilation pathway that could support efficient and sustainable bio-refineries based on highly available feedstock sources, such as electricity.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

890/SMBP

Overproduction of hydrogen-dependent carbon dioxide reductase (HDCR) in the thermophilic acetogenic bacterium *Thermoanaerobacter kivui*

M. Basen*[1], P. Benner[1], I. Geiger[1], H. Dietrich [1], V. Müller[1]
[1]*Goethe University Frankfurt, Molecular Microbiology and Bioenergetics, Frankfurt / Main, Germany*

Introduction: The production of foreign proteins from anaerobic microorganisms such as the thermophilic (TOPT ~65 °C) acetogenic bacterium *Thermoanaerobacter kivui* can be challenging using established expression systems. Many of these proteins are essential for growth or interesting for biotechnological applications, or both, such as the cytoplasmic hydrogen-dependent carbon-dioxide reductase (HDCR). The enzyme was recently purified and characterized from cell-free extracts of the mesophilic acetogenic bacterium *A. woodii* (1). A monomer of the multimeric enzyme consists of 4 subunits: a molybdopterin-containing formate dehydrogenase(FDH), an iron-iron hydrogenase (H_2ase) and two small subunits harboring 4 iron-sulfur clusters each. Due to the nature of its cofactors, the enzyme is highly oxygen-sensitive. The genome of *T. kivui* contains a 5-gene operon putatively encoding a HDCR. We have shown before that *T. kivui* is naturally competent, as reported for other *Thermoanaerobacter* species (2) with transformation frequencies up to $1*10^{-2}$.
Objectives: We aimed to engineer *T. kivui* to overproduce its own HDCR for biochemical studies.
Material and Methods: We used the replicating *Thermoanaerobacterium-E. coli* shuttle plasmid pMU131, conferring resistance to kanamycin at 60 °C (2) as basis to develop a vector for protein production in *T. kivui.*
Results: The HDCR operon of *T. kivui* was fused by PCR to the putatively strong constitutive promoter of the *T. kivui* S-layer protein. A gene sequence encoding a Strep-Tag was introduced at the N-Terminus of the FDH. The fusion product was cloned into pMU131, yielding the 13-kb large vector pPB5, which was subsequently transformed into *T. kivui*. The cell-free extract (CFE) of 10 kanamycin-resistant isolates were screened for FDH activity, and the isolate with the highest activity, strain HDCR10, was chosen for further experiments. CFEs of strain HDCR10, grown on glucose, catalyzed formate-dependent hydrogen production and hydrogen-dependent carbon dioxide reduction with specific activities of 5 U mg^{-1} and 1 U mg^{-1}, respectively, an increase of 30–100 x versus CFEs of the wild type. In the next step, the protein was purified using the Strep-Tag. The purified protein contained subunits of the predicted sizes for all subunits and catalyzed HDCR with a specific activity of 60 U mg^{-1}.
Conclusion: We developed a plasmid-based system for overproduction of proteins in the thermophilic acetogenic bacterium *T. kivui*. As proof-of-principle, we chose the relatively large hydrogen-dependent carbon dioxide reductase, a protein of high biotechnological interest. Active enzyme was produced and purified using a Strep-Tag. In conclusion, we hold in hand an interesting alternative for production and purification of proteins from anaerobes.

References

[1] Schuchmann & Müller (2013). Science 342: 1382-1385.
[2] Shaw *et al.* (2010). Appl Environ Microbiol 76**:**4713-4719.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

891/SMBP

Characterisation of heterologous gene transcription and translation by an *on-line* monitoring system *in vivo*

S. Wagner*[1], M. Ziegler[1], A. Kremling[1], K. Pflüger-Grau[1]
[1]*TU Munich, Systembiotechnology, Garching, Germany*

In biotechnological approaches it is often necessary to introduce a multiplicity of genes into a bacterial host system as e.g. *Escherichia coli*. Such a heterologous expression can cause a competition for limited cellular resources as ribosomes, polymerases, and other precursors. The demand caused by the additional expression of genes in the end leads to a collapse in the production of the desired protein and arrests the cells in growth.
In order to optimise the productivity of a heterologous pathway, it is first necessary to understand the cellular processes during foreign protein production, i.e. to quantitatively analyse the distribution of the resources between the intrinsic processes needed for maintenance and the extra load introduced by the heterologous pathway. To this end, we constructed a plasmid that allows us to monitor and quantify transcription and translation of a desired gene in vivo. This was achieved by fusion of the mCherry encoding gene (reflecting the load) with the RNA-aptamer dBroccoli, which is giving a GFP-like output in combination with the fluorophore DFHBI-1T [1]. The protein level was monitored via the fluorescent protein mCherry.
With this tool, we were able to quantify the mRNA *on-line* without cellular disruption, and got information about transcription (mRNA per gene copy number) and translation rate (protein per mRNA). To get a systematic insight into transcription and translation, we analysed different constructs and induction levels. This allowed us to compare the cellular fitness and resources under different rates.
With this method, we are able to quantify not only mRNA production rates, but also mRNA stability. Because of the easily measurable fluorescence signals, this system has the potential to be a valuable tool for the systematic high-throughput analysis of pathway fine-tuning. Measurements can give information about the expression pattern and distribution of the participating enzymes. Bottlenecks can be identified and, at the end of the day, circumvented or avoided.

References

[1] Filonov, G.S., Kam, K.W., Song, W., Jaffrey, S.R. In-gel imaging of RNA processing using Broccoli reveals optimal aptamer expression strategies, Chemistry & Biology, 22, pp 649–660.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

892/SMBP
Microbial long-chain alkane synthesis from rapeseed oil
S. Maurer*[1], J. Schrader[1], M. Buchhaupt[1], H. Schewe[1], O. Albert[1]
[1]*Dechema Research Institute, Biochemical Engineering, Frankfurt am Main, Germany*

Microbial synthesis of long-chain alkanes and their potential use as drop-in biofuels has been widely investigated in the past years. Most studies focus on a *de novo* approach via the fatty acid biosynthesis pathway by fermentation of simple substrates, such as glucose.
In this work we created a microbial platform that converts domestic oil resources of vegetable origin, e.g. rapeseed oil, to synthesize a mixture of long-chain alkanes and alkenes. An auto-displayed lipase hydrolyzes extracellular triglycerides of rapeseed oil (mainly C16 and C18) to glycerol and free fatty acids. While glycerol serves as carbon and energy source the fatty acids are first reduced to intermediate aldehydes, using an intracellular carboxylic acid reductase and/or an acyl-CoA reductase. Cyanobacterial aldehyde deformylating oxygenases then convert the aldehydes to produce n-1 alkanes and alkenes, which are released into the reaction medium. The microbial platform strain was further streamlined by gene deletions and insertions to balance the dynamic equilibrium of the substrates and intermediates and to direct the flux towards the introduced pathway.
We present different production hosts, namely *P. putida* and *E. coli*, compare different production routes, and demonstrate the efficiency of targeted gene deletions.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

893/SMBP
Understanding CO2 binding and activation in reductive carboxylases
G. Stoffel*[1], T. Erb[1]
[1]*Max Planck Institute For Terrestrial Microbiology, Biochemistry and Synthetic Biology of Microbial Metabolsim, Marburg, Germany*

Question: Nature possesses enzymes which are able to activate and incorporate CO2 into an organisms biomass using simple yet remarkably efficient carboxylation mechanisms. As a case study, we used the recently discovered Crotonyl-CoA carboxylase/reductase (Ccr) [1] which belongs to the medium chain dehydrogenase/reductase (MDR) superfamily. This enzyme carboxylates Crotonyl-CoA to (*2S*)-Ethylmalonyl-CoA oxidizing one equivalent of NADPH and is the fastest carboxylase known today. Here we present the finding of conserved residues involved in CO2 binding/activation and characterization of mutants in these residues. A new method for the assessment of binding of carbon dioxide through 13C-NMR spectroscopy is being developed and will shed light on the specific interactions with the proteins active site.
Methods: X-Ray crystallography was used to identify residues responsible for CO2 interactions in the active site of Ccr. These were confirmed to be conserved among all enzymes of this family by protein sequence alignment. We then kinetically characterized several mutants for these residues using UV/Vis spectroscopy and determined the carboxylation efficiency by HPLC/MS. We are currently developing a 13C-NMR-based method to directly observe 13CO2 in the active site of the enzyme and gain a deeper understanding of its interactions with the enzyme.
Results: The crystal structure of Ccr shows three highly conserved residues namely Asn, Phe/Tyr, Glu, which distinguish carboxylases from reductases in this enzyme superfamily. The kinetic characterization of these enzymes shows that the enzyme is rendered unable to carboxylate upon mutating these residues, an aspect that is also reflected by the HPLC/MS analysis of the reaction products. Preliminary results using a 13C-NMR-based method suggest CO2 binding in the active site.
Conclusions: This study will clarify the yet unknown catalytic principles carboxylases employ for the activation of carbon dioxide and will extend our knowledge on aspects of enzyme catalysis.

References
[1] Erb et al. PNAS 2007

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

894/SMBP
Construction of Corynebacterium glutamicum for the production of anthranilate.
J. W. Youn*[1], S. Behnken[2], J. Magnus[2], G. Jäger[3], G. Sprenger[1]
[1]*University of Stuttgart, Institute of Microbiology, Stuttgart, United Kingdom*
[2]*Bayer AG, Leverkusen, Germany*
[3]*Covestro AG, Leverkusen, Germany*

Introduction: Corynebacterium glutamicum is a versatile workhorse for the industrial white biotechnology. It is mainly used for the industrial production of the amino acids L-glutamic acid and L-lysine. Furthermore it was reported that C. glutamicum can be genetically modified to produce other amino acids, organic acids, sugars and alcohols (1, 2).
Objectives: Anthranilate (ortho-aminobenzoate, o-AB) is the first intermediate of the L-tryptophan biosynthesis pathway in C. glutamicum (3). o-AB is a valuable aromatic compound as it can be used for different applications (4). The aim of this study was to construct C. glutamicum to produce o-AB from a renewable source like glucose.
Methods: The gene of the anthranilate phosphoribosyltransferase (trpD, cg3361) was modified in C. glutamicum. We deleted either the gene trpD or changed the ribosomal binding site (rbs) of trpD. Growth and accumulation of o-AB was determined within the different constructed strains.
Results: The deletion of trpD leads to an auxotrophy for L-tryptophan in minimal media with glucose as sole carbon source. The reintroduction of trpD restored the growth of C. glutamicum ΔtrpD and an accumulation of o-AB could be observed in strains with changed rbs of trpD.
Conclusion: Here we demonstrated that C. glutamicum can be genetically engineered to produce o-AB from glucose. We used a strategy to reduce the activity of TrpD by modifying the rbs of trpD. This modification in C. glutamicum was sufficient to allow growth and accumulation of o-AB in minimal media with glucose as sole carbon source.

References
[1] Wendisch VF. 2016. Microbial Production of Amino Acid-Related Compounds. Advances in biochemical engineering/biotechnology.
[2] Lee JY, Na YA, Kim E, Lee HS, Kim P. 2016. The Actinobacterium Corynebacterium glutamicum, an Industrial Workhorse. J Microbiol Biotechnol 26:807-822.
[3] Matsui K, Sano K, Ohtsubo E. 1987. Sequence analysis of the Brevibacterium lactofermentum trp operon. Molecular & general genetics : MGG 209:299-305.
[4] Kuepper J, Dickler J, Biggel M, Behnken S, Jager G, Wierckx N, Blank LM. 2015. Metabolic Engineering of Pseudomonas putida KT2440 to Produce Anthranilate from Glucose. Frontiers in microbiology 6:1310.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

POSTERSESSION Zoonoses (FG ZO)

895/ZOP

Analysis of heavy metal compounds triggering the success of MRSA CC398 in healthcare settings

S. van Alen*[1], R. Köck[1,2], E. Idelevich[1], G. Peters[1], U. Kaspar[1], K. Becker[1]
[1]*Institute of Medical Microbiology, Universitätsklinikum Münster, Münster, Germany*
[2]*Institute of Hospital Hygiene, University of Oldenburg, Oldenburg, Germany*

Question: In the past 15 years, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) belonging to the clonal complex (CC) 398 emerged in hospital settings. Heavy-metal compounds such as zinc oxide or copper sulphate are being used as feed supplements in livestock for growth promotion and prevention of gastro-intestinal diseases. As part of type V SCC*mec* elements, which are highly prevalent among LA-MRSA CC398, a zinc and cadmium resistance encoding gene was detected. In MRSA CC398, this could lead to decreased heavy-metal susceptibility and selection of MRSA CC398 also in the hospital, where surfaces or dermatologic products often comprise heavy metals. In order to test this hypothesis, resistance patterns of LA-MRSA CC398 against a set of different heavy metal compounds were evaluated and compared to non-LA-MRSA strains.
Methods: The minimum inhibitory concentrations (MICs) of cationic heavy metal ions (zinc chloride, copper sulphate and nickel chloride) were determined for 10 LA-MRSA CC398 isolates, 10 isolates of hospital- (HA) and 10 of community-associated (CA) MRSA clonal lineages. Furthermore, the survival of MRSA CC398 on specific antimicrobial surfaces (copper and zinc coated platelets) was investigated.
Results: MICs of zinc chloride were considerably higher in LA-MRSA (MIC50, 4 mM; MIC90, 8 mM) compared to HA-MRSA (MIC50, 0.5 mM; MIC90, 2 mM) and CA-MRSA (MIC50, 1 mM; MIC90, 2 mM). No differences between LA-MRSA, HA-MRSA and CA-MRSA were found for copper sulphate (MIC50 and MIC90, each 8 mM) and nickel chloride (MIC50 and MIC90, each 8 mM). Applying survival assays, all MRSA survived on copper platelets up to 10 minutes; the survival on zinc platelets was up to 60 minutes. For MRSA CC398, after a six day incubation on copper surfaces, an increased survival after 20 minutes incubation was observed (d6: 3.4%, d7: 0.2%, d8: 6%, d9: 0.8%, d10: 0.3%), whereas HA-MRSA only showed a higher survival on day six (0.5%) and day seven (4%) compared to the growth control.
Conclusions: In summary, heavy metal-compounds might provide a fitness benefit for MRSA CC398 compared to other MRSA lineages and might facilitate survival and transmission of this clonal lineage in the inanimate healthcare environment. The decreased susceptibility of MRSA CC398 to heavy metal compounds (e.g. zinc chloride) could also impact on human colonization, if these compounds are used for treatment (e.g. in dermatology).

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

896/ZOP

Analysis of the Porcine Nasal Culturome with Special Regard to Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) Colonization – An Update

A. Schlattmann*[1], K. von Lützau[1], U. Kaspar[1], G. Peters[1], K. Becker[1]
[1]*University Hospital Münster, Medical Microbiology, Münster, Germany*

Introduction: *Staphylococcus aureus* infections remain one of the major problems in European healthcare institutions, exacerbated by ongoing antibiotic resistance. Livestock-associated methicillin-resistant strains (LA-MRSA) of these bacteria, in particular LA-MRSA CC398, colonize porcine as well as human nasal cavities and pose the source for subsequent nosocomial infections. Elucidation of general bacterial colonization patterns might yield a way to understand how the composition of microorganisms in the porcine nasal habitat influences *S. aureus* carriage.
Objectives: In this work, the culturome – i.e., the viable and cultivable bacteria colonizing the surfaces of pig noses – has been investigated.
Materials and Methods: Eighteen pigs were sampled by taking swab samples from inside the nasal cavity (n = 18) and from the snout surface (n =18). Transport in Amies medium and processing of samples occurred within 24 hours. Dilutions of 1:10, 1:100, and 1:1000 were prepared and 0.1 mL was inoculated onto blood, chocolate, CAP, and MacConkey agar plates and incubated aerobically (Chocolate agar: + 5 % CO_2). Schaedler, Schaedler + K/V, chocolate and CAP agar plates were used for anaerobic incubation. Incubation temperature and time were 35 °C and 48 hours. MALDI-TOF mass spectrometry (MS) analysis was used for identification. Isolates were stored at -80 °C. Distribution of *S. aureus* colonization versus colonization with other bacterial genera and species was tested using Fishers exact test.
Results: Analyzing 36 samples, 152 different bacterial species have been found. Most frequently isolated and identified bacterial species were *Rothia nasimurium* (36/36), *Corynebacterium xerosis* (24/36), *Aerococcus viridans* (23/36), *Escherichia coli* (22/36), *Staphylococcus epidermidis* (20/36), *Streptococcus hyovaginalis* (17/36), *S. aureus*, *Staphylococcus haemolyticus*, *Streptococcus suis* (each 15/36) and *Leuconostoc citreum* (12/36). Statistically significant differences ($p<0.05$) in simultaneous co-colonization negatively correlating with *S. aureus* were obtained for the genera *Citrobacter*, *Lactobacillus*, *Lactococcus*, and *Pseudomonas*, and the species *Lactobacillus curvatus*, *Pseudomonas putida*, and *Staphylococcus equorum* while positively correlating co-colonization was observed for *Clostridium* spp. and *S. hyovaginalis*.
Conclusion: Our data revealed a very complex composition of the porcine nasal microbiota with *Rothia nasimurium* as part of the core culturome. *S. aureus* carriage was detected in approximately one third of the samples and has been found to be associated with simultaneous colonization with certain bacterial genera and species.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

897/ZOP

In vitro* activity of a recombinant chimeric bacteriophage endolysin against livestock associated methicillin resistant *Staphylococcus aureus

U. Kaspar*[1], J. A. De Haro Sautto[1], G. Peters[1], A. S. Scherzinger[2], E. A. Idelevich[1], K. Becker[1]
[1]*University Hospital Münster, Institue for Medical Microbiology, Münster, Germany*
[2]*Hyglos GmbH - a bioMérieux Company, Bernried, Germany*

Introduction: Methicillin resistant *Staphylococcus aureus* strains predominantly associated to the livestock (LA-MRSA) are significantly introduced into human healthcare facilities. Thus, LA-MRSA strains particularly of the clonal complex (CC) 398 present an additional risk factor for human health and burden to

the healthcare system. Apart from classical antibiotic therapy, endolysins from bacteriophages offer alternative treatment options against *S. aureus* without triggering antibiotic selection pressure. The recombinantly produced endolysin HY-133 has been shown to be effective against *S. aureus* but its efficiency against LA-MRSA has not been determined yet. In this work, the *in vitro* activity of endolysin HY-133 was studied against different LA-MRSA either harboring the genes *mecA* or *mecC* as methicillin resistance markers.
Methods: *In vitro* activity of the bacteriophage endolysin HY-133 (Hyglos GmbH, Bernried, Germany) against a representative set of 50 *mecA* LA-MRSA and 35 *mecC* LA-MRSA was evaluated by broth microdilution method according to the CLSI guidelines. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined for all strains using the direct colony suspension method recommended for staphylococci. Furthermore, killing kinetics of HY-133 were analyzed in time-kill-curves against a subset of four LA-MRSA strains (2x *mecA*, 2x *mecC*).
Results: Both groups of LA-MRSA were successfully inhibited in their growth by HY-133 *in vitro* with identical MIC and MBC median values presented within each group. Comparison between *mecA* and *mecC* strains showed similar activities of HY-133 with MIC50/MBC50 values of 0.25 µg/mL for both groups and MIC90/MBC90 values slightly increased in the group of *mecC* LA-MRSA (1 µg/mL) compared to *mecA* strains (0.5 µg/mL). Moreover, ranges of both MIC and MBC values were broader for *mecC* strains (0.12-4 µg/mL) than for *mecA* strains (0.06-1 µg/mL). In time-kill-curve experiments, all concentrations of HY-133 led to a fast decrease of growth rates for LA-MRSA. A bactericidal effect was observed at a 16-fold level of the MIC. However, regrowth of LA-MRSA was observed after a bactericidal effect had been reached.
Conclusion: HY-133 proved to be active against a wide set of different LA-MRSA *in vitro*. MIC/MBC values against LA-MRSA were comparable to those against other MRSA and MSSA strains. Time-kill-curves revealed a fast mode of action of HY-133, but regrowth of cultures was observed *in vitro*. This phenomenon warrants further investigations.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

898/ZOP
Prepare for the rare – fast exchange of database entries for strengthening MALDI-TOF MS diagnostics on the example of Streptobacillus
T. Eisenberg*[1], J. Rau[2]
[1]*Hessisches Landeslabor, Veterinärmedizin, Giessen, Germany*
[2]*Chemisches und Veterinäruntersuchungsamt Stuttgart , Fellbach, Germany*

MALDI-TOF mass spectrometry (MALDI-TOF MS) is a rapidly spreading technique, e.g. within medical microbiology, food control and also in veterinary medical diagnostics. An unknown microorganism can be identified by comparing its mass spectrum to that of a reference database. The systems employed by most users allow for the addition of one"s own data. These can be transferred within the device platform, making it possible to fill in current diagnostic gaps more quickly. We provide examples for user derived reference database entries for *Streptobacillus moniliformis*, the causative agent of zoonotic rat bite fever, and closely related novel species, that we have recently described [1,2].
In order to provide information regarding such own new database entries from users for users, we have set up an open catalog under "*MALDI-TOF-MS-user-platform.ua-bw.de*" [3]. This non-commercial list offers spectra specific information regarding species names, isolate numbers and specimens as well as details on the validity of the isolate designation and technical details of the entries (instrument, cultivation, preparation etc.). Furthermore, the platform offers contact information to the creator of respective spectral entries, but does not intend to provide these for free download. The copyrights of the data and the updating process of the equipment manufacturers will thereby not be affected. The list is open to users under the aforementioned conditions for the purpose of mutual exchange of information.

References
[1] Eisenberg, T., K. Imaoka, M. Kimura, S. P. Glaeser, C. Ewers, T. Semmler, J. Rau, W. Nicklas, T. Tanikawa, P. Kämpfer (2016) *Streptobacillus ratti* sp. nov., isolated from a black rat (*Rattus rattus*). Int J Syst Evolut Microbiol, 66(4):1620-6. doi: 10.1099/ijsem.0.000869. [Epub ahead of print 2015]
[2] Eisenberg, T., W. Nicklas, N. Mauder, J. Rau, M. Contzen, T. Semmler, N. Hofmann, K. Aledelbi, C. Ewers (2015) Phenotypic and genotypic characteristics of members of the genus *Streptobacillus*. PLoS One, 10(8):e0134312.
[3] Rau, J., T. Eisenberg, R. Sting (2015) MALDI-TOF MS User Platform. http://maldi-tof-ms-user-platform.ua-bw.de.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

899/ZOP
NGS-based analysis of AmpC-beta-lactamase CMY-2-producing *Escherichia coli* from humans, livestock and food in Germany
M. Pietsch[1], A. Irrgang[2], N. Roschanski[3], G. Brenner Michael[4], H. Rieber[5], A. Käsbohrer[2,6], S. Schwarz[4,7], U. Rösler[3], Y. Pfeifer*[1], G. Werner[1]
[1]*Robert Koch Institute, FG13 Nosocomial Pathogens and Antibiotic Resistance, Wernigerode, Germany*
[2]*German Federal Institute for Risk Assessment (BfR), Department of Biological Safety, Berlin, Germany*
[3]*Freie Universität Berlin, Institute for Animal Hygiene and Environmental Health, Berlin, Germany*
[4]*Friedrich-Loeffler-Institut , Institute of Farm Animal Genetics, Neustadt-Mariensee, Germany*
[5]*Medizinisches Versorgungszentrum Dr. Stein, Division of Microbiology, Mönchengladbach, Germany*
[6]*Veterinary University Vienna, Vienna, Austria*
[7]*Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany*

Introduction: Resistance to third-generation cephalosporins in *Escherichia coli* is mainly mediated by extended-spectrum beta-lactamases (ESBLs) and AmpC-beta-lactamases. Overexpression of the naturally, chromosomal-located *ampC* gene of *E. coli* causes cephalosporin resistance, but more common are plasmid-encoded AmpC enzymes (e.g. CMY, ACC, DHA) that were acquired from other species. The most frequent AmpC enzyme is CMY-2. It is produced by ca. 1% and ca. 30% of the third-generation cephalosporin-resistant *E. coli* from humans and poultry, respectively.
Objectives: To identify possible pathways of transmission of the bla_{CMY-2} gene or CMY-2-producing *E. coli* clones, we performed whole-genome sequencing of 170 isolates collected between 2008 and 2016 all over Germany in the scope of different studies of the national research project "RESET".
Materials & Methods: Genomic DNA of CMY-2 positive *E. coli* from different sources (humans n=51, healthy broilers n=51, chicken meat n=56, turkey meat n=7, diseased pigs/chickens n=5,) was extracted and sequenced using the Illumina MiSeq platform. Reads were assembled by A5 algorithm. Resistance genes and phylogenetic markers, such as multi-locus sequence type and plasmid replicon types were identified (CGE Finder series).
Results: The 170 sequenced isolates showed a highly diverse distribution of sequence types (STs) and replicon types. Fifty-nine different STs were identified; most prevalent types were ST38

(n=19) as well as ST131 (n=16) and ST117 (n=13). The highest intersection of STs between the different reservoirs were found for ST131 (human n=8/food n=2/animal n=6) and ST38 (3/9/7). Frequent plasmid replicon types were FIB (n=138) and FII (n=90), IncI1 (n=87) and IncK (n=80). Analyses of the *bla*CMY-2 containing contigs revealed the replicon types IncK (n=74) and IncI1 (n=62) as the gene bearing plasmidic backbone for most of the isolates. Additional beta-lactamase genes (bla_{TEM}, bla_{CTX-M}, bla_{OXA}, bla_{SHV}) were detected in 50% of the isolates; and 12 *E. coli* from broilers and retail chicken meat carried the colistin resistance gene *mcr-1*.

Conclusions: The results showed clonal relatedness for CMY-2-positive *E. coli* from different origins for the clonal lineages ST131 and ST38. Frequent correlation of a plasmid replicon type to distinct STs was shown for IncK and ST57, ST429 and ST38. In contrast, IncI1 was associated with all seven ST58 isolates. However, the majority of isolates belonged to various clones and harbored different bla_{CMY-2}-bearing plasmids. This indicates a more likely plasmid-mediated spread rather than a clonally driven spread of bla_{CMY-2} across the *E. coli* host populations.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

900/ZOP

Tuberculosis among Swiss captive elephants: microevolution of *Mycobacterium tuberculosis* characterized by MLVA genotyping and WGS

G. Ghielmetti*[1], U. Friedel[1], S. Gagneux[2]
[1]*University of Zurich, Institute of Veterinary Bacteriology, Zurich, Switzerland*
[2]*Swiss Tropical and Public Health Institute, Basel, Switzerland*

Mycobacterium (M.) tuberculosis was cultured from several organs, including lung tissue and gastric mucosa, of three captive elephants euthanized in a Swiss zoo. The elephants presented weight loss, weakness and exercise intolerance. Serological testing with DPP® VetTB Assay for Elephants (Chembio Diagnostic Systems, USA) suggested an infection with a member of the *M. tuberculosis* complex. Despite numerous ante-mortem negative trunk wash investigations, in two out of three elephants *M. tuberculosis* was isolated from pharyngeal and trunk mucosa. Shedding of the pathogen was therefore confirmed. Preliminary molecular characterization of the obtained isolates by spoligotyping revealed an identical profile, namely SIT276, suggesting a single source of infection. Multi-locus variable number of tandem repeat analysis (MLVA) elucidated two divergent populations of bacteria and mixed infection in one elephant, suggesting either different transmission chains or prolonged infection over time. A total of eight isolates cultured from different organs were submitted for whole genome sequencing (WGS) analysis, confirming a single source of infection, presumably a zookeeper. The present findings demonstrate that the epidemiological application of MLVA for *M. tuberculosis* isolates from elephants is questionable. Further investigations including accurate ante-mortem diagnostic tests are needed to prevent the spread of *M. tuberculosis* among elephants and animals of the same holder as well as transmission to humans.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

901/ZOP

Birds and deer in a zoological garden in Germany infected by different *Mycobacterium avium* subsp. *avium* strain

P. Möbius*[1], G. Wibbelt[2], I. Moser[1], H. Köhler[1], C. A. Szentiks[2]
[1]*Friedrich-Loeffler-Institut (Federal Research Institute for Animal Health), Institute of Molecular Pathogenesis, Jena, Germany*
[2]*Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany*

Members of the species *Mycobacterium* (*M.) avium* belong to the non-tuberculous mycobacteria (NTM) and are distributed in the proximate and more distant human environment. They have been isolated from soil and surface water worldwide, but also affect many wildlife and livestock species. *M. avium* subsp. *hominissuis* (MAH) is an opportunistic pathogen for humans, swine and other mammals. *M. avium* subsp. *avium* (MAA) is the causative agent of avian tuberculosis. MAA was also isolated from humans in countries with close contact between man and poultry.

The aim of this study was to assess which mycobacterial species can be isolated from clinically diseased birds and mammalians in an individual zoological garden and if epidemiological links can be unveiled.

35 tissue samples suspicious for mycobacterial infection originating from 13 diseased animals belonging to 7 avian and 3 mammalian species of a zoological garden in Germany were investigated. Mycobacterial species and subspecies were identified by specific PCR reactions targeting *hsp*65, IS*1245* and IS*901*. Isolates were genotyped by multi-target genotyping using MIRU-VNTR and IS*901*-RFLP-analysis.

All isolated bacteria belonged to the *Mycobacterium avium* complex. MAA could be isolated from 20 samples of 10 individual animals belonging to 6 avian species (Cröllwitz turkey hen [*Meleagris gallopavo f. domestica*], Northern hawk owl [*Surnia ulula*], Blue ear pheasant [*Crossoptilon auritum*], Sickle duck [*Anas falcata*], Wonga pigeon [*Leucosarcia melanoleuca*], Greaterwhite-fronted goose [*Anser albifrons*]) and Buchara deer [*Cervus elaphus bactrianus*]. MAH was isolated in addition to MAA from a kidney of the goose; the goose was simultaneously infected by these two subspecies. Altogether, the isolates belonged to 5 combined MAA genotypes: Turkey hen, duck, and pigeon were infected by genotype 1, hawk owl and pheasant by genotype 2, deer isolates from 3 animals by genotype 3, and genotype 4 and 5 was found in a goose, suggesting a mixed infection. Genotyping results suggest different infection sources (e.g. free-ranging wild birds), and a putative animal to animal transmission of MAA.

As susceptible zoo animals and the soil of the enclosures could be a potential source of NTM infection for humans, close contact should either be avoided or prompt thorough hygiene measures especially implemented when handling animals in zoological gardens.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

902/ZOP

The immunoglobulin binding protein EibG in Shiga toxin producing *Escherichia coli* (STEC) is highly stable and the expression is regulated comprehensive

D. Rubin[1], H. Karch[1], T. Kuczius*[1]
[1]*Institute for Hygiene, Westfälische Wilhelms-University and University Hospital Münster, Münster, Germany*

Shiga toxin (Stx)-producing *Escherichia coli* (STEC) which harbour *eib*G genes synthesize immunoglobulin binding proteins in *E. coli* (Eib). These EibG proteins described as virulence factors bind to human immunoglobulins in a non-immune manner. The regulation of EibG expression is highly complex. STEC produce high protein levels under static growth while shearing stress generated by shaking during growth repressed EibG

production. Further effects of an up-regulation were reduced oxygen tension and increased incubation temperatures.
To find out additional environmental factors for up- and down-regulation of protein synthesis, we studied EibG expression in various media, analysed protein stability after prolonged incubation, and we compared the protein levels with several STEC.
STEC were cultivated in complex medium (LB) and low-grade media as yeast extract and minimal medium without and with shaking (180 rpm) at 37°C for 24 to 96 h. After pelleting by centrifugation cells were lysed and proteins were separated by SDS-PAGE followed by immunoblotting and EibG signal visualization using human IgG Fc fragment as detection antibody. Phenotypic appearance was analysed macro- and microscopically.
Despite diverse EibG levels in various isolates, STEC produced commonly high EibG amounts in complex medium and lower levels in low-grade and minimal media under static growth conditions. This regulation seems related to other *E. coli* strains which carry other *eib* genes as well. Microscopically, STEC exhibited chain formation and aggregation in all analysed media, while aggregates were only visible after static growth in complex medium. When EibG is synthesized in high amounts, the proteins exhibited high stability even during prolonged incubation over days.
Our findings indicate firstly that Eib protein synthesis is regulated comprehensive with an outcome of different expression levels in STEC isolates and that positive up-regulation conditions result in distinct phenotypes and secondly that EibG proteins demonstrate high stability during prolonged incubation.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

903/ZOP
Characterization of opportunistic pathogens of the nasal cultural microbiome of dog and cat owners vs. other participants of a German general population cohort
R. Koeck*[1], F. Schaumburg[1], C. Fegeler[2], A. W. Friedrich[3], K. Becker[1]
[1]*University Hospital Münster, Institute of Medical Microbiology, Münster, Germany*
[2]*University of Heilbronn, GECKO Institute of Medicine, Informatics and Economy, Heilbronn, Germany*
[3]*University of Groningen, UMCG, Department of Medical Microbiology, Groningen, Netherlands*

Introduction: In Germany, companion animals such as dogs and cats are kept in more than one third of all households. This might lead to a zoonotic transmission of (multi-resistant) bacteria between pets and their human owners.
Objectives: In this study, we compare nasal carriage of *Staphylococcus aureus*, enterobacteria and selected nonfermentative gram-negative bacteria among dog and cat owners from the German general population with persons who do not keep these pets.
Patients & methods: In a cohort of 1,878 adult persons from North Rhine-Westphalia and Lower Saxony, we identified dog and cat owners by questionnaire. Every participant provided at least one nasal swab; consecutive nasal swabs were collected 6-8 and 12-14 months after the initial sampling. Swabs were cultured on Columbia blood agar and MacConkey agar as well as *S. aureus* and ESBL-selective agars after non-selective enrichment. All *Staphylococcus aureus*, enterobacteria, *Acinetobacter* sp. and *Pseudomonas* sp. isolates were characterized by MALDI-TOF mass spectrometry and antibiogram (VITEK-2). *S. aureus* were *spa* typed; MRSA was confirmed by *mec*-PCR. Various risk factors were assessed by questionnaire and univariate statistical analysis was performed using Chi-Square or Fisher-Exact test.
Results: Of all participants 364 (19%) owned dogs and 277 (15%) cats, respectively. Among these, 84 (5%) owned both.
Comparing dog /cat owners vs. other people showed significant ($p<0.05$) differences for the following risk factors: living in one-person-households (8%/12% vs. 19%), occupational contact with livestock (5%/8% vs. 1%), smoking (36%/38% vs. 27%) and neurodermitis (5%/7% vs. 3%). Dog owners also used oral antibiotics (25% vs. 19%) more frequently. Nasal carriage of *S. aureus*, enterobacteria and nonfermenters overall did not differ significantly, except for the carriage of *Pantoea agglomerans* (6% vs. 2%) among dog owners.
MRSA carriage rates were 0.4% among dog owners and 1% among cat owners, but did not differ significantly from the controls. *S. aureus* isolates carried by dog owners were more resistant to clindamycin and erythromycin ($p=0.001$). The top 5 *spa* types were t091, t084, t008, t012 and t002 among dog owners and t084, t091, t002, t012 and t015 among cat owners, respectively.
Conclusion: Culturing different bacteria from dog and cat owners and comparing carriage rates to other persons yielded no relevant differences except an increased colonization rate with *Pantoea agglomerans* and increased lincosamide/macrolide resistance among *S. aureus* isolates from dog owners.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

904/ZOP
Shiga toxin 2 binds antithrombin and heparin: Ancillary condition or key event in Escherichia coli-induced HUS (eHUS)?
M. Berktold[1], L. Knabl[1], D. Orth-Höller[1], R. Würzner*[1]
[1]*Med. Univ. Innsbruck, Div. Hygiene & Med. Microbiology, Innsbruck, Austria*

Hemolytic uremic syndrome (HUS) is a life-threatening complication of infection with Shiga toxin (Stx) producing Escherichia coli and characterized by thrombocytopenia due to platelet consumption. However, the pathogenesis of this Escherichia coli-induced HUS (eHUS) is still unresolved.
In the present study the interactions between Stx, platelets and the plasmatic coagulation were investigated. FACS analyses and live microscopy did not show any binding of Stx to platelets and neither activation nor aggregation of platelets by Stx could be observed. Functional coagulation tests with ROTEM using platelet-poor plasma, however, revealed that increasing Stx concentrations led to a decreased clotting time, indicating an activation of plasmatic coagulation with quicker onset of clot formation. Subsequent tests employing ELISA and Co-immunoprecipitation demonstrated a significant binding affinity between Stx and antithrombin, one of the most important inhibitors of blood coagulation. Interestingly, this binding affinity of Stx to antithrombin was influenced by the pH, being more pronounced at lower pH values. Quartz crystal microbalance with dissipation monitoring (QCM-D) clearly confirmed binding of Stx to heparin and showed an impaired binding of antithrombin to Stx-bound heparin. In contrast, no binding between Stx and protein C, thrombin or blood coagulation factor Xa was observed.
In summary, the results of this study showed a significant effect of Stx on plasmatic coagulation, which may contribute to platelet consumption and may explain the low success of heparin therapy in eHUS patients.

Presentation: Tuesday, 7 March 2017 from 16:00 - 18:00 in the Poster Foyer.

C

D

G

H

L

M

N

O

P

Q

R

S

T

X

Y

Z